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THE LACTOSE AND CHLORIDE CONCENTRATIONS OF MILK PRODUCED DURING IRREGULAR INTERVALS BETWEEN MILKINGS[1]

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In conducting routine analyses of milk for lactose in connection with experiments on the influence of the components of milk upon its taste, the authors observed slight variations in the lactose content of the milk produced at consecutive milkings from individual cows. The samples in certain instances were collected after unequal intervals between milkings, a fact which suggested the possibility that the interval between milkings might have an influence upon the lactose and chloride concentrations of the milk. It has previously been shown (8) that the ratio of chloride per cent to lactose per cent in milk is one of the most important relations between the components of milk influencing its primary taste. It was believed that a study of the effect of unequal intervals between milkings upon the lactose and chloride content of milk would be a contribution to our knowledge of the factors affecting the primary taste of milk.

REVIEW OF LITERATURE

Workers in the New York Agriculture Experiment Station at Geneva (4), conducting an experiment on the effect of the stage of lactation on the chemical and physical composition of milk, during the period 1890–1894, concluded that next to the ash, sugar was the least variable constituent of milk. Eckles and Shaw (1), conducting a similar experiment at a later date, came to the same conclusion and further stated that the sugar is strikingly uniform as compared with the protein and fat. They (2) later concluded, from their experiments on the influence of the breed and the individual on milk, that the average lactose per cent is constant for the breed, but that individuals, noticeably the Holsteins, show considerable variation from the average. Other work by Eckles and Shaw (3) on the variations in milk from individual cows shows that while sugar is usually considered to be one of the least variable constituents of the milk produced by the individual cow it may be expected to vary rather more than the total protein. They report that a variation of 0.5 per cent from the average is not uncommon, but state that 90 per cent of the samples show a variation of less than 0.2 per cent.

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[1] Presented at the annual meeting of the American Dairy Science Association, Ames, Iowa, June 25, 1930.

[2] Acknowledgment is made to the Animal Husbandry Division for the use of animals for this experiment.

Eckles and Shaw (3) have reported slight variations in the lactose content of milk produced at consecutive milkings when the intervals between milkings were equal. Jackson and Rothera (6) have shown that normally there are slight variations in the conductivity of the milk from consecutive milkings, the evening milk generally having the higher conductivity. Taylor (9) and Coste and Shelborn (10) attribute 49 to 79 per cent of the conductivity to chloride ions. They also show that there is no apparent relation between electrical conductivity and ash content or the solids-not-fat content of milk. Therefore, Jackson and Rothera's data may be interpreted to mean that normally there are slight variations in the chloride content of milk from consecutive milkings. Jackson and Rothera, however, do not state the interval between milkings. The writers (7) have found that feeding molasses and sodium chloride to four cows did not significantly change the lactose and chloride content of the milk when the cows were milked at 12 hour intervals. The writers found no work reported in which the interval between milkings was made the variable.

PROCEDURE

Two cows, each in the second month of lactation, which produced milk with a pleasing taste were selected from the Station herd. Throughout the experiment a standard ration consisting of a grain mixture, eight pounds of green alfalfa and alfalfa hay *ad libitum* was placed before the cows immediately after milking. All feed was consumed by the cows throughout the experiment, except some of the hay, which was removed from the mangers five hours before milking. This procedure was followed in order to avoid feed flavor in the milk since samples were also examined for taste. Weights of feed consumed and milk produced were recorded for each milking and samples were collected for analyses and taste.

The animals were milked at 12 hour intervals for a preliminary period of 5 consecutive days. This was followed for 5 consecutive days by the experimental period of unequal intervals between milkings, consisting of 14 hours between night and morning milkings and 10 hours between morning and night milkings. The animals were then returned to the original equal intervals between milkings for a period of 5 days.

Lactose was determined by the Official Munson and Walker Method (11) with the following modification: the cuprous oxide produced was determined by the potassium permanganate titration of Bisson and Sewell (12). Chloride was determined by a modification of the Volhard Method (13). Fat was determined by the Babcock Method.

In recording the taste scores, the milk score card of the United States Department of Agriculture was used. Milk scoring 13.5 or higher was considered above criticism. The judging was done by two experienced judges working independently.

Table 1 shows a slight variation in the yield of milk, the percentage of fat, and the percentages of lactose and chloride during equal intervals between milkings. During the unequal intervals between milkings the yield and fat percentages showed wide variations, while the lactose and chloride percentages remained practically the same as during the period of equal intervals between milkings. It will be observed that the taste scores remained uniform throughout the experiment. Table 1 applies to cow 421, while figure 1 shows a graph prepared from the data secured from cow 607.

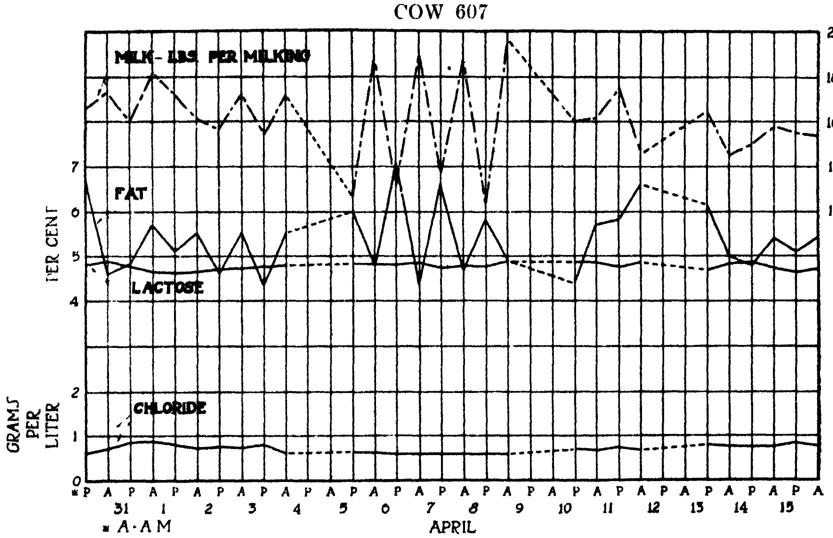

Fig. 1. The Yield and Partial Composition of Milk in Relation to the Interval Between Milking.

The preliminary period of equal intervals between milkings is shown from March 31 to April 4. The experimental period of unequal intervals from April 5 to April 9, inclusive, and the period of return to equal intervals between milkings from April 10 to April 15, inclusive, are represented. During the entire experiment, the lactose percentage and chloride in grams per liter were not significantly changed. It is to be noted, moreover, that the total yield of lactose varied directly with the variation in yield of milk. The taste of the milk produced by cow 607 was uniform and without criticism throughout the experiment.

DISCUSSION OF RESULTS

A study of the data presented in the table and graph indicates that although there was a trend of slightly higher lactose percentages and slightly lower chloride concentrations during the period of unequal intervals between milking, the changes were so slight that they may be considered insignificant when compared with the marked variations in the

TABLE 1

Cow 421. The yield, partial composition and taste of milk produced at equal and unequal intervals between milkings

INTERVAL BETWEEN MILKING	DATE	A. M.						P. M.					
		MILK	LACTOSE	CHLORIDE	TASTE SCORE	FAT	TOTAL LACTOSE	MILK	LACTOSE	CHLORIDE	TASTE SCORE	FAT	TOTAL LACTOSE
		Lbs.	Per cent	Grams per liter		Per cent	Grams	Lbs.	Per cent	Grams per liter		Per cent	Grams
EQUAL	3/30/30							14.4	4.83	0.68	13.4	5.7	315
	3/31	15.8	4.77	0.69	13.5	4.5	343	16.1	4.80	0.71	13.5	5.2	351
	4/1	14.7	4.72	0.72	13.5	5.1	318	14.0	4.61	0.75	13.5	5.6	293
	4/2	13.0	4.75	0.68	13.5	5.7	279	16.6	4.81	0.68	13.5	7.4	361
	4/3	14.0	4.84	0.64	13.5	5.4	307	15.4	4.81	0.63	13.5	5.9	336
	4/4	15.8	4.83	0.64	13.5	5.4	347						
UNEQUAL	4/5							13.3	4.86	0.66	13.5	7.6	293
	4/6	16.1	4.88	0.63	13.5	4.7	355	11.3	4.84	0.66	13.5	7.9	273
	4/7	19.3	4.84	0.60	13.5	5.8	421	13.0	4.80	0.61	13.5	7.9	283
	4/8	16.6	4.89	0.60	13.5	4.7	367	12.8	4.76	0.60	13.5	7.2	305
	4/9	18.0	4.85	0.60	13.5	5.6	395						
EQUAL	4/10							15.0	4.75	0.72	13.5	4.3	324
	4/11	14.9	4.79	0.68	13.5	5.8	324	14.6	4.89	0.75	13.5	4.3	324
	4/12	15.8	4.76	0.75	13.5	4.7	340						
	4/13							15.4	4.76	0.73	13.5	6.1	322
	4/14	14.6	4.76	0.71	13.5	5.6	315	14.6	4.74	0.74	13.5	5.7	313
	4/15	13.0	4.78	0.74	13.5	5.9	325	15.7	4.77	0.75	13.5	5.8	339
	4/16	14.4	4.78	0.74	13.5	5.9	312						

yield of milk and in the percentages of milk fat produced during this period.

It has long been recognized that unequal intervals between milkings cause wide variations in the percentages of fat in the milk, the shorter the interval the higher the fat percentage. This is probably to be expected in the light of recent studies by Peterson, Palmer and Eckles (14) in their work on the problem of the synthesis and secretion of milk fat, in which they concluded that the milk fat is secreted by a separate mechanism from that responsible for the secretion of the other ingredients of milk.

CONCLUSIONS

(1) Unequal intervals between milkings did not significantly affect the lactose and chloride concentrations of the milk produced by two cows in this experiment.

(2) No change in the taste of the milk was observed during the course of the experiment.

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THE EFFECT OF PASTEURIZATION UPON BRUCELLA MELITENSIS VAR. SUIS

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In a paper presented by Dr. Walter W. Lee[1] at the sixty-sixth annual meeting of the American Veterinary Medical Association it was stated that prevention of undulant fever in human beings is simplicity itself and that no person need have the disease if he will but avail himself of the means of prevention; namely, use no milk that has not been pasteurized. In the discussion following, some question was raised by various speakers as to the efficacy of pasteurization as commonly applied, particularly upon the porcine strain of the Brucella organism which certain investigators had reported was not, as is the case with the bovine, killed by a temperature of 140°F. for 15 minutes. The desirability of more information on the subject of pasteurization as it affects the Brucella group led to the experiments described in this paper.

Boak and Carpenter[2] report experiments of Park which showed that a mixed culture of Brucella abortus isolated from cattle and swine was killed by exposure of 140°F. for 10 minutes, 142°F. for 7 minutes and 145°F. for 5 minutes.

These same authors report the results of thermal death point studies of several strains of Br. abortus of bovine, porcine and human origin grown in milk. The cultures were exposed in Sternberg bulbs submerged for varying intervals in an electrically controlled water bath. Human and bovine cultures were destroyed in 15 minutes at 140°F. No cultures were viable after 20 minutes when tested by injection into guinea pigs and by inoculation on suitable media. They[3] also state that the cultures most virulent for guinea pigs showed the higher thermal death point and that the porcine strain was most resistant. Other investigators have reported similar results but none in America so far as indicated by available literature have made the tests using the type of pasteurizer in common use by the dairy industry.

Proescholdt[4] has shown that B. abortus (Bang) is killed by heating ½ hour at 60°–65°C. (140°–149°F.). Zeller and Wedemann[5] have conducted experiments to test the effect of continuous pasteurization upon milk in-

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[1] Jour. A. V. M. A., LXXV (1920) pp. 679–690.

[2] Jour. Inf. Dis., 43 (1928), No. 4, pp. 327–329.

[3] Jour. Bact., 15 (1928), No. 1, p. 41.

[4] Molkerei Zeitung, 40 (1926), p. 461.

[5] Arb. aus dem Reichgesundheit, 61 (p. 1).

oculated with Br. abortus (bovine). Five experiments were carried out, using an Ahlborn pasteurizer of two types, one with a preheater and the other without. This Ahlborn type pasteurizer is very similar to the machines in common use in creameries and pasteurizing plants in America. It consists of a wood vat, metal lined and equipped with a tubular rotating stirrer, through which water heated by steam is continually flowing to maintain the desired temperature of the milk batch. It is equipped with a recording thermometer to register the milk temperature throughout the process. The type equipped with preheater differs from the other only in that a separate container is installed to retain the milk while it is being brought to the desired temperature. As soon as this temperature is reached the milk is conducted into the pasteurizer proper where the temperature is maintained under constant agitation.

Thirty different bovine Bang strains, some freshly isolated, others cultured in the laboratory for a long time were used. In each experiment the washings of growth from 18 to 30 Kolle dishes, incubated 2 to 10 days were filtered through a single paper filter and added to 250 to 550 liters of milk. Varying degrees of heat were used for different times and cultures were made in bouillon and upon common and Drigalski agar. Guinea pigs were inoculated in different ways with the pasteurized samples. After two months the pigs were autopsied and cultured. In most of the tests run the bacteria were all killed in the milk after it had passed the preheater (to bring it to 60°C. before placing it in the pasteurizer proper). In a few cases the organisms were still viable after passing the preheater but when the 60°C. temperature was maintained for 10 minutes all were destroyed. The conclusion, stated by the investigator is, that continuous pasteurization at 60°-63°C. (140°-145.4°F.) for ½ hour is sufficient to destroy all Bang abortion (bovine) organisms contained in milk.

EXPERIMENTAL

The experiments above reported were so conclusive as pertains to the bovine strain that it seemed unnecessary to repeat them but of more importance to conduct tests using the porcine strain of Brucella which, as shown by reference to the literature, is more heat resistant than the bovine However, as checks on our own work two batches of milk inoculated with the bovine strain were run. The apparatus used was a 100 gallon pasteurizer kindly loaned us by the Dairy Industry Department. It was set up in a room of the laboratory where water, steam and electric connections were possible. The machine (see Fig. 1) is a coil machine known by the manufacturer as Class A-1. It was equipped with a recording thermometer. In the first 5 runs made the temperature as indicated on the chart of the recording thermometer never showed below 60°C. (140°F.) and never above 62.2°C. (144°F.). To check the accuracy of this ther-

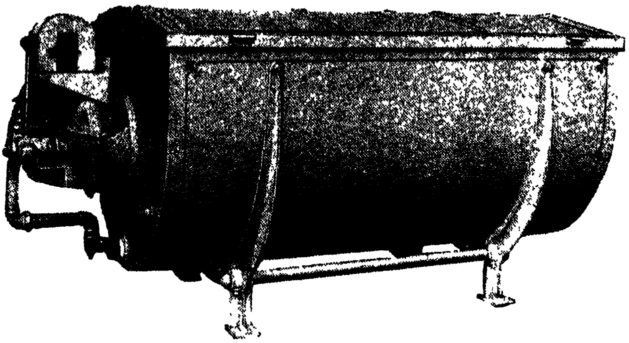

FIG. 1. VAT PASTEURIZER USED IN THE EXPERIMENTS

mometer, temperatures were read on a 100°C. standardized laboratory thermometer suspended in the pasteurizing vat at a point about 6 inches from the place of insertion of the recording thermometer. The latter showed no temperature below 61.5°C. (142.7°F.) nor above 63°C. (145.4°F.); differences as compared with the record on the recording thermometer of 2.7° to 1.4°F.

The results of the first 5 runs were irregular and unsatisfactory. Test No. 1 showed all organisms killed in a sample removed after 1 minute exposure at 63°C. (145.4°F.) while tests 2, 3, 4 and 5 showed viable organisms in samples removed after exposure up to 62°C. for 4 minutes and in the foam and outlet after 30 minutes up to 63°C. In seeking a cause for this irregularity we enlisted the help of those familiar with commercial pasteurizing methods and learned that advice given us when the machine was being set up was erroneous. This was to the effect that the lid of the pas-

EXPERIMENT 1—(July 9, 1930)

Cultures:—Porcine 4, 44, 51. Four agar slants each, 48 hours old, washed off with milk, filtered through glass wool and mixed with milk

Milk:—Fifteen gallons pasteurized, not clarified, temperature 15°C. placed in vat and raised to 60°C. in 7 minutes. Machine operated with open lid

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 8–12		Lesions 8–12		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	15°C.	0	0	0	0	0	0	0
Inoculated	15°C.	Mold	400	400	+	+	+	+
After 0 min.	60°C.	“	400	400	+	+	+	+
“ 1 “	63°C.	“	0	0	0	0	0	0
“ 2 “	63°C.	“	0	0	0	0	0	0
“ 3 “	63°C.	“	0	0	0	0	0	0
“ 4 “	63°C.	“	0	0	0	0	0	0
“ 5 “	62.5°C.	“	0	0	0	0	0	0
Outlet after 30 minutes		“	0	0	0	0	0	0
Foam after 30 minutes		“	0	0	0	0	0	0

EXPERIMENT 2—(Aug. 14, 1930)

Cultures:—Same as Experiment 1

Milk:—Same as Experiment 1, raised from 12°C. to 60°C. in 12 minutes. Lid open

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 9-23		Lesions 9-23		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	12°C.	0	0	0	0	0	0	0
Inoculated	12°C.	+	Died	400	+	+	+	+
After 0 min.	60°C.	+	400	400	+	+	+	+
" 1 "	61.7°C.	Mold	Died	400	0	+	0	+
" 2 "	62°C.	"	0	400	0	0	0	+
" 3 "	62.5°C.	"	0	400	0	+	0	+
" 4 "	62°C.	"	400	400	+	+	+	+
" 5 "	62°C.	"	0	0	0	0	0	0
Outlet after 30 minutes	62.5°C.	"	400	400	+	+	+	+
Foam after 30 minutes	62.5°C.	"	200	400	0	0	0	0

teurizer need not be closed and acting upon this, contrary to our better judgment, our first 5 runs had been made with an open lid.

The next 4 runs were made with a closed lid and, as indicated in the charts, were uniform and satisfactory. The cultures used in all experiments except 8 and 9 are the same, and consist of 3 porcine strains, No. 4 isolated in 1926, No. 44 isolated in August 1929 and No. 51 isolated in February 1930. All are of tested virulence for guinea pigs. The quantity of culture used was that obtained from 4 agar slants of each strain, 12 in all, grown 48 hours, washed off with milk and then filtered through glass wool. These cultures were added to 15 gallons of pasteurized milk, not clarified. Samples of milk to be tested were removed from the pasteurizer

EXPERIMENT 3—(Oct. 1, 1930)

Cultures:—Same as Experiment 1

Milk:—Same as Experiment 1, raised from 12.5°C. to 60°C. in 13 minutes. Lid open

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 10–30		Lesions 10–30		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	12.5°C.	0	0	0	0	0	0	0
Inoculated	12.5°C.	+	400	400	+	+	+	+
After 0 min.	60°C.	0	400	400	+	+	+	+
" 1 "	61.5°C.	Mold	Died	200	0	+	0	+
" 2 "	61.5°C.	0	400	400	+	+	+	+
" 3 "	61.5°C.	0	400	0	+	0	+	0
" 4 "	61.5°C.	Mold	400	0	+	+	+	0
" 5 "	61.5°C.	0	200	200	+	0	+	+
Outlet after 30 minutes		0	400	400	+	+	+	+
Foam after 30 minutes		0	200	25	0	0	+	0

EXPERIMENT 4—(Oct. 14, 1930)

Cultures:—Same as Experiment 1

Milk:—Same as Experiment 1, raised from 10°C. to 60°C. in 9½ minutes. Lid open

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 11-14		Lesions 11-14		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	10°C.	Mold	0	0	0	0	0	0
Inoculated	10°C.	"	400	400	+	+	+	+
After 0 min.	60°C.	"	400	400	+	+	+	+
" 1 "	62°C.	"	400	400	+	+	+	+
" 2 "	62°C.	"	400	400	+	+	+	+
" 3 "	62°C.	"	400	400	+	+	+	+
" 4 "	62°C.	"	400	400	+	+	+	+
" 5 "	61.5°C.	"	400	400	+	+	+	+
Outlet after 30 minutes		"	400	400	+	+	+	+
Foam after 30 minutes		"	0	400	0	+	0	+

at varying intervals with a 10 cc. pipette. The samples were immediately placed in the refrigerator, temperature 10°–14°C. and allowed to stand two or more hours until the cream line formed. Intraperitoneal injections of the cream into guinea pigs and inoculations on agar were made. In most cases the latter were unsatisfactory, showing a heavy overgrowth with molds. Agglutination tests against an antigen prepared from the three porcine strains used for inoculation were conducted on the blood from all injected guinea pigs at intervals of approximately 30 days after injection.

The results of pasteurization in experiment 1 with open lid indicate that all organisms were destroyed after the temperature of 63°C. (145.4°F.) was maintained for 1 minute, not only in samples removed from the vat

EXPERIMENT 5—(Nov. 2, 1930)

Cultures:—Same as Experiment 1

Milk:—Same as Experiment 1, raised from 6°C. to 61.5°C. in 11 minutes. Lid open

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 12-2		Lesions 12-2		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	6°C.	0	0	0	0	0	0	0
Inoculated	6°C.	+	400	400	+	+	+	+
After 0 min.	61.5°C.	None	None	None				
" 1 "	62°C.	0	400	400	+	+	+	+
" 2 "	62°C.	Mold	400	400	+	+	+	+
" 3 "	62°C.	"	400	400	+	+	0	+
" 4 "	62.5°C.	"	0	0	0	0	0	0
" 5 "	62.5°C.	"	0	0	0	0	0	0
Outlet after 30 minutes		"	0	400	0	+	0	+
Foam after 30 minutes		"	400	400	+	+	+	+

EXPERIMENT 5a—(Dec. 12, 1930)

Culture:—No. 20 bovine

Milk:—Same as Experiment 1, raised from 9°C. to 62°C. in 9½ minutes. Lid open as in Experiment 5

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 1–9–31		Lesions 1–9–31		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	9°C.	None	0	0	0	0	0	0
Inoculated	9°C.	"	400	400	+	+	+	+
After 1 min.	62°C.	"	100	25	+	0	+	0
" 2 "	61.5°C.	"	400	400	+	0	+	0
" 3 "	61.5°C.	"	400	400	+	+	+	+
" 4 "	61.7°C.	"	25	200	0	0	+	0
" 5 "	62.2°C.	"	0	0	0	0	0	0
Outlet after 30 minutes		"	400	400	+	+	+	+
Foam after 30 minutes		"	0	0	0	0	0	0

of the pasteurizer but also in those from the outlet and from the foam. In the other 4 tests with open lid, results were variable. In no case was the temperature range (60°–63°C. for 30 minutes) sufficient to kill the organisms from the foam or outlet samples. The outlet consists of a 1 inch faucet with the cutoff at the lower end. The milk contained in this was not agitated by the stirrer and consequently probably never reached even the minimum temperature of the milk in the vat. Certain it is that the organisms contained were still viable, as evidenced by the infection set up in 9 of the 10 guinea pigs inoculated. In Experiment 5a which is a check test substituting a bovine strain for the porcine strains used in the first 5, results differed only in that living organisms were not obtained from the

EXPERIMENT 6—(Dec. 8, 1930)

Cultures:—Same as Experiment 1

Milk:—Same as Experiment 1, raised from 12.5°C. to 61°C. in 7½ minutes. Lid closed except when samples taken. Outlet plugged from inside

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 1–5–31		Lesions 1–5–31		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	12.5°C.	0	0	0	0	0	0	0
Inoculated	12.5°C.	+	Dead	400	+	+	+	+
After 3 min.	62.7°C.	0	0	0	0	0	0	0
" 8 "	63°C.	0	0	0	0	0	0	0
" 15 "	62.7°C.	Mold	0	0	0	0	0	0
" 20 "	62.3°C.	0	0	0	0	0	0	0
" 25 "	62.5°C.	0	0	0	0	0	0	0
" 30 "	62.7°C.	0	0	0	0	0	0	0
Foam		0	0	0	0	0	0	0

EXPERIMENT 7—(Dec. 10, 1930)

Cultures:—Same as Experiment 1

Milk:—Same as Experiment 1, raised from 13°C. to 61°C. in 11 minutes. Lid closed and outlet plugged

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 1-8-31		Lesions 1-8-31		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	13°C.	Mold	0	0	0	0	0	0
Inoculated	13°C.	‘‘	400	400	+	+	+	+
After 3 min.	62°C.	‘‘	0	0	0	0	0	0
‘‘ 8 ‘‘	62.8°C.	‘‘	0	0	0	0	0	0
‘‘ 15 ‘‘	62.5°C.	‘‘	0	0	0	0	0	0
‘‘ 20 ‘‘	63°C.	‘‘	0	0	0	0	0	0
‘‘ 25 ‘‘	62.5°C.	‘‘	Dead	0	0	0	0	0
‘‘ 30 ‘‘	62.5°C.	‘‘	0	0	0	0	0	0
Foam		‘‘	0	0	0	0	0	0

foam. This suggests that the porcine strains used may be slightly more heat resistant than the bovine. In any case, pasteurization with open lid for 30 minutes is not effective in destroying either the porcine or bovine varieties of Brucella.

In Experiments 6, 7 and 7a slight changes in technique were made. The lid was kept closed except while sampling and the outlet of the machine was closed with a rubber stopper on the inside, thus giving an effect similar to the use of the usual flush valve. Temperature readings were made from a standardized thermometer inserted through a rubber stopper in the recording thermometer opening into the vat. To check these readings a second thermometer was inserted through an opening in the lid to a point

EXPERIMENT 7a—(Jan. 7, 1930)

Culture:—Bovine 20

Milk:—Same as Experiment 7, raised from 7°C. to 61°C. in 9 minutes. Lid closed and outlet plugged

SAMPLE	TEMP.	CULTURES	GUINEA PIGS					
			Titer 2-2-31		Lesions 2-2-31		Cultures	
			Pig 1	Pig 2	Pig 1	Pig 2	Pig 1	Pig 2
Uninoculated	9°C.	None	0	0	0	0	0	0
Inoculated	9°C.	‘‘	400	0	+	+	+	0
After 5 min.	62.5°C.	‘‘	0	0	0	0	0	0
‘‘ 10 ‘‘	61.5°C.	‘‘	0	0	0	0	0	0
‘‘ 15 ‘‘	62.5°C.	‘‘	0	0	0	0	0	0
‘‘ 20 ‘‘	62°C.	‘‘	0	0	0	0	0	0
‘‘ 25 ‘‘	62°C.	‘‘	0	0	0	0	0	0
‘‘ 30 ‘‘	62.5°C.	‘‘	0	0	0	0	0	0
Foam		‘‘	0	0	0	0	0	0

just above the surface of the milk, to determine the effect of opening and closing the lid for sampling at the intervals indicated in Experiment 6. While the lid was kept closed the difference between the temperature of the milk and that of the air above was not over 2°C. When the lid was opened to obtain samples the temperature of the air fell as much as 8°C. but was restored in 1 minute to at least 61°C. In these three tests pasteurization was effective in 3 minutes at 62°C. (143.6°F.) so far as milk tests show and in 30 minutes as indicated by tests of the foam. It is more than probable it was effective for the foam in less than 30 minutes but animal inoculations were not made with foam before this time. It was not demonstrated that pasteurization with the lid closed would be effective for the milk in the outlet but from the results of the first 6 experiments it would not appear probable, since the milk contained in the faucet would not be more agitated with a closed lid than with an open one and the milk in this dead space would probably never reach the temperature necessary for effective pasteurization.

SUMMARY

Pasteurization tests, using a standard pasteurizing outfit show that a temperature of 62°–63°C. (143.6°–145.4°F.) applied 3 minutes is sufficient to destroy Brucella organisms, both the bovine and porcine varieties. This indicates that the usual pasteurization temperature of 62°–63°C. for 30 minutes gives an ample factor of safety, providing the pasteurizer is operated in the proper manner. With the lid of the pasteurizer open a much longer time exposure to this temperature is required and the results obtained are irregular and uncertain, in that viable organisms were recovered from the foam even after 30 minutes pasteurization. The absolute necessity of a flush gate valve is also indicated, for in those experiments in which an ordinary faucet outlet was used, viable organisms were obtained from the outlet after 30 minutes pasteurization whereas with the outlet closed by a stopper on the inside, no living organisms remained after 3 minutes exposure. The experiments emphasize the importance of carefully conducted operations in pasteurization if successful results are to be obtained.

THE VITAMIN A, $B(B_1)$ AND $G(B_2)$ CONTENTS OF MILK THROUGHOUT THE YEAR[1]

FLORENCE L. MACLEOD, JESSIE B. BRODIE AND EMILY R. MACLOON

The vitamin value of milk has frequently been shown to vary with the feed which the cow is receiving. Kennedy and Dutcher (1) in 1922 concluded from experiments on both stall- and pasture-fed cows that stall-fed cows will produce a milk rich in vitamins A and B provided their ration consists of a proper combination of grains and leafy foods.

Drummond and his associates (2), Luce (3), Chick and Roscoe (4), and Golding, Soames and Zilva (5), have shown that the vitamin A content of milk is raised by the feeding of cod-liver oil, fresh pasture or kale while the vitamin D content is higher when the cows are on pasture in the sun or when receiving a comparatively large quantity of cod-liver oil.

Milk has been found to be relatively richer in vitamin $G(B_2)$ than in vitamin $B(B_1)$ by Sherman and Axtmayer (6), Hunt and Krauss (7) and Outhouse, Macy, Brekke and Graham (8).

Bechdel, Honeywell, Dutcher and Knutsen (9) reported experiments in 1928 which showed that the vitamin B complex may be synthesized by bacteria in the rumen of the cow and, therefore, that the amount in the milk is not always dependent on the amount in the ration.

Our purpose in the experiments to be reported here, was to determine the vitamin A, $B(B_1)$ and $G(B_2)$ values of milk from one dairy of stall-fed cows at all times of the year. In previous work from this laboratory (10) it has been shown that the vitamin C value of milk from the same dairy is high and remains constant throughout the year. The cows which furnished the milk received a ration containing an alfalfa hay of excellent quality, a good ensilage, and a concentrate.

VITAMIN A CONTENT

Series I

The first series of experiments on the vitamin A value of the milk was carried out during the year July, 1926, to July, 1927, using the method described by Sherman and Munsell (11). The diet (laboratory No. 379A) devoid of vitamins A and D but adequate in other respects had the following composition:—purified casein, 18 per cent; cornstarch, 67 per cent; dried brewery yeast, 10 per cent; Osborne and Mendel salt mixture, 4 per

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cent; and sodium chloride, one per cent. In this series the pre-experimental store of vitamin D was depended upon to carry the animals through both the depletion period and the experimental period of closely restricted growth.

Rats, 28 to 29 days old, which had been raised on Diet B of this laboratory, containing whole milk powder one-third by weight and whole wheat two-thirds by weight, were placed on the diet described above and were continued on it until they ceased to grow. The animals were then placed in separate cages with raised screen bottoms and were given measured amounts of milk. One animal from every litter was continued on the basal diet with no milk as a negative control.

The animals of this series were fed amounts of milk, varying in quantity from 0.25 cc. to 1.0 cc. per day, six days in the week.

It can be seen from Table I that at all seasons of the year a unit gain in weight (a gain of approximately 3 grams per week for the experimental period) was obtained when 0.5 cc. of milk per day was fed six days in the week. No seasonal variation in the vitamin A content of the milk was found, the gains for the eight-week periods during the summer, fall, winter and spring being 25.2, 26.3, 27.2 and 27.4 grams, respectively.

TABLE 1

Growth of rats fed a Vitamin A-free diet (Diet 379A) only or this diet plus graded amounts of milk at different seasons of the year

SEASON	AMOUNT OF MILK FED PER DAY	NUMBER OF ANIMALS	AV. GAIN IN 8 WKS. AND P. E.	REMARKS
	cc.		*grams.*	
Summer	0	10		Av. survival 28.9 days
Fall	0	9		Av. survival 25.8 days
Winter	0	15		Av. survival 31.0 days
Spring	0	8		Av. survival 28.3 days
Summer	0.25	15		Av. survival 39.7 days
Summer	0.5	11	25.2 ± 1.6	Killed end exp. period
Fall	0.5	14	26.3 ± 1.2	Killed end exp. period
Winter	0.5	15	27.2 ± 1.7	Killed end exp. period
Spring	0.5	11	27.4 ± 2.2	Killed end exp. period
Summer	0.75	16	34.6 ± 2.1	Killed end exp. period
Fall	0.75	20	33.2 ± 1.3	Killed end exp. period
Winter	0.75	16	33.8 ± 1.2	Killed end exp. period
Spring	0.75	4	33.2 ± 0.9	Killed end exp. period
Fall	1.0	20	42.3 ± 1.3	Killed end exp. period
Winter	1.0	5	38.4 ± 1.0	Killed end exp. period

On autopsy thirty-five per cent of the animals receiving 0.5 cc. of milk showed signs of vitamin A deficiency such as pus in the tongue and submaxillary glands while 16 per cent receiving 0.75 cc. and 8 per cent receiving 1.0 cc. showed these signs.

Series II

During 1928 to 1929 much of the work was repeated providing vitamin D in the basal diet in the form of irradiated commercial cholesterol. Half of the animals in each litter used were given the original basal diet while the other half received vitamin D in addition. No differences were found in the gains made in the experimental periods by the animals on the two diets. In this series, a unit gain in weight was made when 0.75 cc. of milk per day was fed, the fourteen animals which received an extra source of vitamin D gaining 25.5 grams and the same number receiving the basal diet without vitamin D, 24.1 grams. The milk, therefore, was a slightly poorer source of vitamin A in 1928 to 1929 than in 1926 to 1927.

From all the results obtained on the vitamin A value of the milk in both series it can be definitely concluded that animals receiving 0.5 to 0.75 cc. per day of the milk six days a week will gain about 3 grams a week for the eight weeks' experimental period at any time of the year. The milk contains, therefore, at least 600 units of vitamin A per pound or 1.3 to 2 units per gram. Dutcher, Honeywell and Dahle (13) found that 1 cc. of raw milk per day, as the only source of vitamin A, produced a gain of 3 grams a week in rats. The cows furnishing the milk received very little fresh pasture. Outhouse, Macy and co-workers (8) found, when feeding milk similar to that used in these experiments, that 3 cc. per day were required to give approximately normal growth. Supplee and Dow (14) reported that 1.65 cc. of milk per day gave a growth in rats of "2.16 to 3.75 grams per week," the first determination being made in January and the second in April. The milk examined in the experiments reported here is, therefore, a rich as well as a uniform source of vitamin A.

VITAMIN B(B_1) CONTENT

The method here used for the measurement of the vitamin B(B_1) values was that worked out by Chase (15). The experimental diet (laboratory No. 513) recommended by Chase (15) consisted of:—purified casein, 18 per cent; Osborne and Mendel salt mixture, 4 per cent; butter fat, 8 per cent; cod liver oil, 2 per cent; cornstarch, 53 per cent; and autoclaved baker's yeast, 15 per cent.[2] Litters of young rats which had been raised to 28 or 29 days of age on Diet B of this laboratory *ad libitum* plus raw beef at a level of 30 grams per animal per week were given the diet devoid of vitamin B and were continued on this diet until they were stationary in weight. The animals were then placed in individual cages and fed milk in graded amounts. One animal from each litter was continued on the basal diet as a negative control.

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[2] This "baker's" yeast was of relatively low initial vitamin B content and was autoclaved for 6 hours at 15 pounds pressure and 120°C., in the presence of 0.1 M potassium hydroxide.

The quantities of milk fed were 4, 8, 10 and 12 cc. per day, six days per week, with three to seven animals on each level during every season. The 4, 8 and 12 cc. levels were fed at all seasons of the year, the 10 cc. level only in the winter and spring.

The variation in the gains made during the year by animals receiving the same quantity of milk is not great and it is a question whether the differences found from season to season are significant. The animals receiving 4 cc. of milk per day lost more weight in the summer than at any other time of the year. In the fall and winter, however, there were six cases of polyneuritis and three deaths among the animals receiving 4 cc. of milk. In the spring none of the animals showed any signs of polyneuritis and their average loss in weight during the 8-week period was only 5.5 grams as compared with losses of 18.7, 12.5 and 11.4 grams in the summer, fall and winter, respectively. Judging from these results it might be concluded that the milk was richest in vitamin B in the spring.

The average gains made by animals receiving 8 cc. of milk were 16.5, 29.4, 29.3 and 33.2 grams for the summer, fall, winter and spring, respectively. This rate of gain was found by Chase (15) to be most satisfactory in testing for vitamin B. The results obtained in the summer showed very little variation and were definitely poorer than those obtained for the other seasons. The gains made by the animals in the spring were slightly better than those made in the fall or winter.

The average gains made by the animals receiving 10 cc. of milk per day in the winter and spring were 39.7 and 46.5 grams, respectively, a slightly larger gain, therefore, being made in the spring.

The animals receiving 12 cc. of milk per day made average gains of 51.7, 47.0, 58.2 and 55.5 grams in the summer, fall, winter and spring, respectively. At this level of feeding there is a tendency for the milk to show an improvement in its vitamin B content in the winter as well as in the spring.

There was little variation in the controls, receiving the basal diet only, from season to season, the average lengths of survival for the summer, fall, winter and spring being 22.0, 21.2, 25.8 and 20.3 days, respectively.

In figure 1 the average results for the entire year are given.

When the average results for the year are considered it is found that a unit gain in weight, a gain of about 3 grams a week for the 8 weeks' experimental period, was secured when 8 cc. of milk per day, 6 days in the

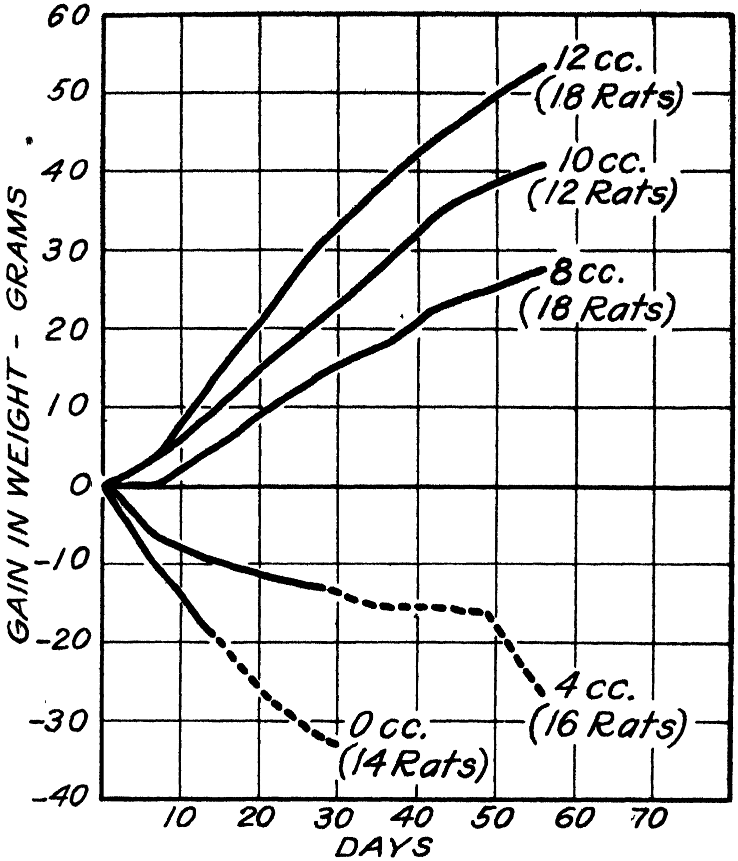

FIG. 1. VITAMIN B (B_1) CONTENT OF MILK

Average gain curves of rats fed milk as sole source of vitamin B during the year July, 1929, to July, 1930. Amount of milk fed per day is shown at end of each curve. Broken line indicates death of one or more rats.

week were fed. Slightly more milk than this would probably have been required in the summer and slightly less in the spring. Ten to fifteen times as much milk is required to give a unit gain in vitamin B experiments as in vitamin A experiments. Milk has, therefore, about 0.1 unit of vitamin B(B_1) per gram, or about 55 units per pound.

VITAMIN G(B_2) CONTENT

The experiments on the vitamin G(B_2) and vitamin B(B_1) values of the milk were carried out simultaneously during the year July, 1929, to

July, 1930. The method used for the determination of vitamin G(B_2) was worked out in this laboratory by Bourquin (16). Rats, 28 or 29 days of age, which had been raised on Diet B of this laboratory *ad libitum,* plus fresh raw beef to the extent of 30 grams per rat per week were fed the following diet (laboratory No. 554) :—purified casein, 18 per cent; Osborne and Mendel salt mixture, 4 per cent; butter fat, 8 per cent; cod-liver oil, 2 per cent, and corn starch, 68 per cent, part of which carried the 80 per cent alcoholic extract from 50 grams of wheat for each 100 grams of diet. The animals were kept on this diet without any additional source of vitamin G until they were constant in weight. They were then placed in individual cages and fed milk in graded amounts in addition to the basal diet. One animal from each litter was continued on the basal diet as a negative control.

The quantities of milk fed were 1, 2, 4 and 6 cc. per day, six days a week. The 6 cc. level was not given in the summer but was fed in the other seasons.

The vitamin G(B_2) content of the milk was found to vary little from season to season. The results obtained on the 2 cc. level in the summer were lower than those found for this level of feeding in the other seasons. The gains made by the animals receiving 1 cc. of milk per day were 4.3, 2.8, 7.8 and 10.0 grams for the summer, fall, winter and spring, respectively, and for the animals receiving 2 cc. the gains were 8.6, 18.6, 22.7 and 21.6 grams for the same seasons, respectively.

The gains made by the animals receiving 4 cc. of milk were of the order which has been found most satisfactory for determinations of this type. The animals receiving this level in the summer, fall, winter and spring gained 34.8, 37.0, 35.7 and 46.5 grams, respectively. The increase in the average gain made by these animals in the spring is probably not significant, for at this same time the controls, receiving the basal diet only with no added source of vitamin G(B_2) made slight gains in weight during the experimental period. The negative control animals lost 10.0, 12.2 and 9.8 grams during the summer, fall and winter, respectively, and gained in the spring 6.2 grams.

In figure 2 the average results for the entire year are given.

These results are nearly identical with those of Bourquin (16) who fed equivalent amounts of skimmed milk powder. In both sets of experiments, it was found that the gains made by the animals increase approximately in proportion to the levels of milk fed.

In order to obtain a gain in weight of about 25 grams in the eight weeks' experimental period, it would probably be necessary to feed about 3 cc. of milk per day, or 4 to 6 times as much as it required to produce the same

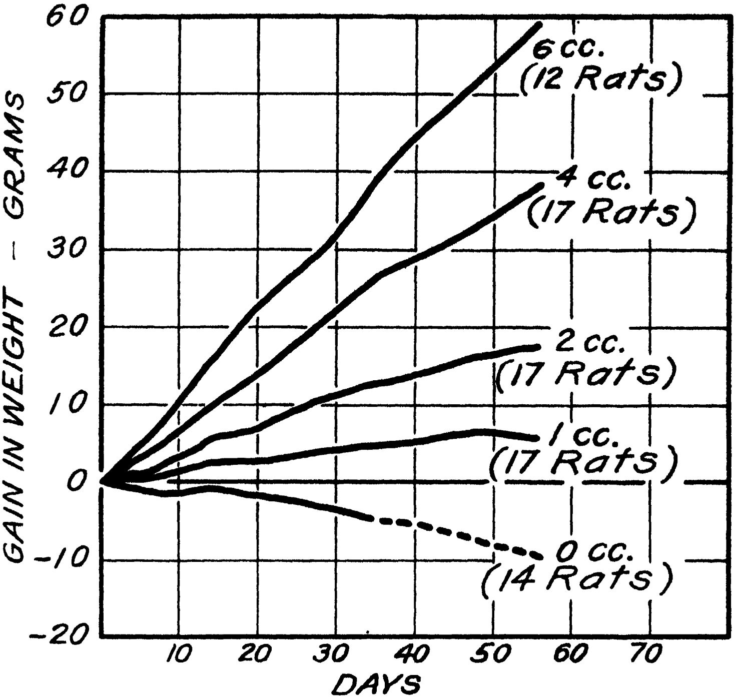

FIG. 2. VITAMIN G (B_2) CONTENT OF MILK

Average gain curves of rats fed milk as sole source of vitamin G during the year July, 1929, to July, 1930. Amount of milk fed per day is shown at end of each curve. Broken line indicates death of one or more animals.

gain in weight in vitamin A experiments. Milk, therefore, contains about 150 units of vitamin G(B_2) per pound, or 0.3 unit per gram, and is, therefore, a good source of this vitamin.

SUMMARY AND CONCLUSIONS

The vitamin A, B(B_1) and G(B_2) values of raw milk have been determined at all seasons of the year.

It was found that the vitamin A value of the milk is comparatively constant from season to season and that 0.5 to 0.75 cc. of the milk per day fed six days in the week will give a unit gain in weight (a growth of 3 grams per week throughout the eight weeks' experimental period) at any time in the year. The milk contains, therefore, about 1.3 to 2 units of vitamin A per gram.

When 8 cc. of milk per day were fed 6 days in the week, as the only source of vitamin B(B_1), an average gain in weight of 3 grams a week for the experimental period (unit gain) was secured. The milk contains, therefore, about 0.1 unit of vitamin B(B_1) per gram.

The vitamin $G(B_2)$ value of the milk varied little from season to season. When 2 cc. of milk per day were fed as the only source of vitamin $G(B_2)$ there was an average gain for the eight weeks' experimental period of 17.8 grams and when 4 cc. were fed there was an average gain of 38.5 grams. To secure a unit gain in weight approximately 3 cc. per day would have to be fed. The milk contains, therefore, about 0.3 unit of vitamin G per gram.

The results reported here, together with those published in an earlier paper (10), indicate that a milk which is produced under excellent conditions of stall-feeding is a uniform and good source of vitamins A, $B(B_1)$, C and $G(B_2)$.

The authors are indebted to Professor H. C. Sherman for the advice given by him during the course of this work.

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THE PHYSIOLOGICAL EFFECT OF RATIONS RESTRICTED PRINCIPALLY OR SOLELY TO THE ALFALFA PLANT

III. THE INFLUENCE OF VARIOUS MINERAL SUPPLEMENTS ON THE CALCIUM, PHOSPHORUS AND NITROGEN METABOLISM OF DAIRY CATTLE[1]

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Attention has previously (1) been called to the calcium, phosphorus and nitrogen metabolism of dairy cattle restricted principally or solely to alfalfa hay. It was shown that dairy cattle whose daily milk yield varied from 9 to 18 kgm. were usually in positive calcium balance; in negative nitrogen balance when the hay contained less than 2.0 per cent nitrogen; and always in negative phosphorus balance when only alfalfa hay was fed. The negative phosphorus balances were changed to slightly positive ones by feeding di-sodium phosphate. In addition to the metabolism trials just referred to, another series was conducted during the summer of 1928, the results of which are reported in this paper.

THE 1928 METABOLISM TRIALS

In these trials we fed second cutting alfalfa hay with and without supplements of di-sodium phosphate, calcium carbonate or bone flour. The hay was grown under irrigation in eastern Oregon and was graded as U. S. No. 1, leafy. According to our analyses, this hay contained 2.06 per cent nitrogen, 1.16 per cent calcium and 0.153 per cent phosphorus. These are average figures, based on the analyses of eight samples as shown in table 1. We again used the two Ayrshire cows which had been used in the 1927 (1) trials. These cows are briefly described in table 2.

Feeding records just previous to the 1928 trials showed that these cows were consuming approximately 32 pounds of alfalfa hay daily. They were therefore offered this amount of chopped hay just previous to and during the metabolism trials. After a careful check on the hay consumption while the cows were becoming accustomed to the metabolism stalls, a series of metabolism trials was conducted in successive units of 5 days each. The

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TABLE 1

Composition of alfalfa hay used in 1928 metabolism trials

SAMPLE NUMBER	NITROGEN	CALCIUM	PHOSPHORUS
	per cent	*per cent*	*per cent*
65	2.00	1.12	.150
66	2.08	1.18	.155
67	2.07	1.20	.163
68	2.04	1.15	.150
69	2.07	1.18	.155
70	2.05	1.14	.153
71	2.10	1.18	.150
72	2.06	1.15	.150
Averages	2.06	1.16	.153

TABLE 2

Description and history of cows used in the 1928 metabolism trials

COW NUMBER	APPROXIMATE AGE	WEIGHT JUNE 18–23	LACTATION PERIOD	PREVIOUS FEEDING HISTORY
	years	*pounds*		
456	6¾	1022	Fifth period began Mar. 26, 1928	On alfalfa hay from Jan. 1925, except for mineral supplements fed during the 1927 metabolism trials.
462	6½	988	Fifth period began May 27, 1928	

mixed excreta and the milk were collected and sampled daily and composite samples analyzed separately for each 5 day period. Instead of discarding the intermediate periods, we included them in order to get a continuous picture of what took place when the mineral contents of the rations were changed. All results are reported as daily averages obtained from consecutive 5 day periods.

The mineral supplements were chosen to check our previous (1) work and with the hope that some light might be thrown on the significance of the high Ca/P intake ratio incident to rations restricted largely to alfalfa. The various mineral supplements were supplied in approximately equivalent amounts, that is, the calcium and phosphorus supplied by the calcium carbonate and di-sodium phosphate, respectively, were roughly equal to the amounts of these elements supplied by bone flour.

DISCUSSION OF RESULTS

The metabolism data are reported in table 3. It will be noted that the nitrogen balances were sometimes positive and sometimes negative. The extent to which the nitrogen balances were influenced by the total protein intake, the biological value of alfalfa crude protein, and the monoto-

TABLE 3

Average daily data secured from 1928 trials

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN FEED MILK EXCRETA BALANCE	CALCIUM FEED MILK EXCRETA BALANCE	PHOSPHORUS FEED MILK EXCRETA BALANCE
			kgm.	*grams*	*grams*	*grams*
456	June 18–23	14.35 kgm. alfala hay	12.04	296.9 50.0 235.2 +11.7	167.6 12.2 166.5 –11.1	22.0 9.4 14.4 –1.8
456	June 23–28	14.47 kgm. alfalfa hay	12.33	298.3 51.2 246.7 +0.4	168.4 11.8 163.1 –6.5	22.1 9.6 13.7 –1.2
462	June 18–23	14.46 kgm. alfalfa hay	15.13	298.2 66.4 235.6 –3.8	168.3 16.8 159.3 –7.8	22.1 12.6 12.0 –2.5
462	June 23–28	14.45 kgm. alfalfa	15.05	298.0 63.5 239.8 –5.3	168.2 16.1 162.2 –10.1	22.1 12.0 12.4 –2.3
456	June 28–July 3	14.47 kgm. alfalfa hay; 160 gm. $CaCO_3$	12.46	298.3 50.7 238.6 +9.0	231.6 11.8 203.4 +16.4	22.1 9.2 14.6 –1.7
456	July 3–8	14.48 kgm. alfalfa hay; 160 gm. $CaCO_3$	11.86	298.5 47.5 245.4 +5.6	231.7 11.1 227.4 –6.8	22.2 8.7 14.7 –1.2
456	July 8–13	14.48 kgm. alfalfa hay; 160 gm. $CaCO_3$	11.71	298.5 46.4 256.1 –4.0	231.7 11.0 232.6 –11.9	22.2 8.4 15.6 –1.8
456	July 13–18	14.47 kgm. alfalfa hay; 160 gm. $CaCO_3$	11.47	298.1 47.5 251.1 –0.5	231.5 10.9 229.2 –8.6	22.1 8.3 15.6 –1.8
456	July 18–23	14.46 kgm. alfalfa hay; 400 gm. di-sodium phosphate	11.25	298.2 45.7 250.1 +2.4	168.3 10.6 170.7 –13.0	56.2 8.2 34.4 +13.6
456	July 23–28	14.23 kgm. alfalfa hay; 400 gm. di-sodium phosphate	10.51	295.2 41.9 247.7 +5.6	166.8 10.0 160.1 –3.3	55.8 7.5 47.0 +1.3

TABLE 3 (*Continued*)

COW NUMBER	DATE	DISTINGUISHING FEATURES OF RATIONS USED	MILK YIELD	NITROGEN FEED MILK EXCRETA BALANCE	CALCIUM FEED MILK EXCRETA BALANCE	PHOSPHORUS FEED MILK EXCRETA BALANCE
			kgm.	*grams*	*grams*	*grams*
456	July 28–Aug. 2	14.47 kgm. alfalfa hay; 400 gm. di-sodium phosphate	10.62	298.2 42.8 245.1 +10.3	168.4 10.5 162.8 –4.9	56.2 7.6 47.4 +1.2
456	Aug. 2–7	14.43 kgm. alfalfa hay; 400 gm. di-sodium phosphate	10.55	297.5 44.1 250.0 +3.4	168.0 10.4 161.4 –3.8	56.1 7.6 47.6 +.9
462	June 28–July 3	14.50 kgm. alfalfa hay; 210 gm. bone flour	14.75	298.7 62.2 245.0 –8.5	234.1 15.9 192.5 +25.7	52.8 11.9 24.3 +16.6
462	July 3–8	14.44 kgm. alfalfa hay; 210 gm. bone flour	13.50	297.9 55.3 233.5 +9.1	233.1 14.3 204.1 +14.7	52.5 10.7 34.9 +6.9
462	July 8–13	14.34 kgm. alfalfa hay; 210 gm. bone flour	12.50	296.6 50.4 250.3 –4.1	231.5 13.2 209.4 +8.9	52.0 9.9 37.4 +4.7
462	July 13–18	14.14 kgm. alfalfa hay; 210 gm. bone flour	11.81	293.0 48.0 260.6 –15.6	221.9 12.4 218.6 –9.1	48.3 9.4 40.2 –1.3

nous and bulky nature of the ration, cannot be determined from this study. The liberal nitrogen intake and the fact that the crude proteins of alfalfa leaves have been shown (2) to be deficient (for rats) in cystine leads us to question the biological value of the proteins of rations consisting largely or entirely of alfalfa hay. It may also be urged that the negative nitrogen balances encountered in this and our previous (1) study may have been due to a low intake of total digestible nutrients. The cows used in the 1928 trials maintained a practically uniform intake (30–32 lbs.) of alfalfa hay during the entire year. This quantity of hay probably did not furnish total digestible nutrients for the production of more than about 30–35 pounds of milk. These cows were in excellent condition before calving. Just after calving, cows 456 and 462 weighed 1093 and 977 pounds respectively. Their milk production never exceeded approximately 40 pounds and averaged about 35 pounds for the first month of lactation. At the beginning of the metabolism trials, these cows weighed 1022 and 988 pounds, respectively. We have been impressed with the small loss in weight during early lactation and are led to wonder if this fact, together with the very rapid decline in milk flow, may not indicate a lack of specific nutrients rather than of total digestible nutrients.

On alfalfa hay only, the calcium and phosphorus balances were negative, in contrast with our previous study (1) in which the calcium balances were usually positive., Feeding di-sodium phosphate again resulted in slightly positive phosphorus balances. The feeding of bone meal resulted in distinctly positive calcium and phosphorus balances. The supplement of calcium carbonate did not appear to affect the storage of either calcium or phosphorus. Apparently the storage of calcium was conditioned by the relatively low phosphorus intake. The failure of calcium carbonate in depressing phosphorus retention apparently means that a Ca/P intake ratio of 10.5 was no more detrimental than one of 7.6. This does not necessarily mean that such ratios are without detrimental effects, but does indicate that under certain conditions at least such ratios may be quite high without producing nutritional disaster. It seems to us, as has been pointed out elsewhere (3), that too many current discussions of this problem fail to take into account the real meaning of the *theory of physiologically balanced salt solutions*. Whether the phosphorus in alfalfa would have been more efficiently utilized in the absence of a possible excess of calcium cannot be determined from our data. Incidentally, the question might also be raised as to whether a supply of calcium 3 to 4 times that called for by the Kellner (4) standard should not be considered as excessive.

The calcium and phosphorus balances are of particular interest because of the rather unusual degree of consistency among the results for successive 5 day periods. The one period which appears to be distinctly out of line is the July 13–18 period for cow 462. This apparent discrepancy is probably explained by the fact that this cow went off feed a few days later. The calcium and phosphorus balances obtained from what might be considered 5 day intermediate periods, in harmony with previous (1) (5) studies, indicate a lag of about 2 days in the excretion of calcium and phosphorus.

We are inclined to interpret this and previous (1) (2) studies as indicating that where cattle are restricted largely to alfalfa, attention can profitably be given to the choice of supplements which will increase the phosphorus content of the ration and improve the biological value of the proteins. Where milk production above perhaps 25–35 pounds is expected, attention should also be given to securing a sufficient intake of total digestible nutrients. The latter statement is based on the assumption that prevailing feeding standards furnish a rough index of the amount of alfalfa hay needed to cover the requirements for maintenance and milk production. As pointed out elsewhere in this paper, we are inclined to hold to the opinion that the rapid decline in milk flow on rations consisting largely of alfalfa hay is suggestive of a lack of specific nutrients rather than of total digestible nutrients. If the latter were the case, it would seem that cows with an inheritance for high production would show a more decided tendency to keep up their milk flow at the expense of body weight.

SUMMARY

We have reported a series of metabolism studies in which the nitrogen, calcium and phosphorus balances of dairy cattle were determined for 16 five-day periods, during which the daily milk yields varied from approximately 10.5 to 15 kgm. The rations fed consisted of alfalfa hay with and without supplements of di-sodium phosphate, calcium carbonate or bone flour.

The nitrogen balances were sometimes positive and sometimes negative. Feeding di-sodium phosphate resulted in slightly positive phosphorus balances. Feeding bone flour resulted in positive calcium and phosphorus balances. A supplement of calcium carbonate did not appear to affect the storage of either calcium or phosphorus.

The relation of these studies to the practice of restricting dairy cattle principally to alfalfa has been discussed in this paper.

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FACTORS AFFECTING THE BODY AND TEXTURE OF PROCESSED CHEESE[1]

HUGH L. TEMPLETON AND H. H. SOMMER

Package processed cheese has become a very important product in the cheese industry. The quality of this product may be affected by a number of factors, yet there has been a marked lack of information concerning them in the literature (1, 2, 3 and 4). A study of the patent literature may give an outline of the general procedure that is used and certain of the added constituents, but it does not give any indication of the factors that control quality. Undoubtedly each manufacturer has made numerous practical observations in his plant, but these are not generally available. The experimental work here reported is part of a study undertaken to contribute to our knowledge of this relatively recent product.

The experimental work has concerned itself mainly with the factors that affect the physical properties of processed cheese. The flavor of processed cheese, as in ordinary cheese, is recognized as an important element in its quality, but an experimental study of flavor is of little permanent value because it is largely controlled by the blending of various lots of cheese. Observations on flavor were therefore made only to indicate any changes which may be attributed to the factors under consideration.

A previous paper (2) has reported observations on the body and texture of processed cheese as affected by (1) "emulsifiers," (2) processing temperature, (3) moisture content, (4) reaction, and (5) the age of the cheese used. The present paper gives further observations on these factors and in addition, on the effect of variations in (6) common salt content and (7) casein-to-fat ratio.

The processed cheese that was made to study the above factors was prepared in a small steam jacketed kettle equipped with double action agitators patterned after kettles used commercially. The full capacity of the kettle was sixteen pounds, but in the experimental work only eight pounds were used because this amount was adequate for samples and could be handled more conveniently. To facilitate accurate control of the processing temperature a special thermometer was built into the hollow agitator shaft, the bulb being at right angles to the shaft and revolving in the cheese. (For further details see previous paper).

In a previous paper (2) it had been found that cheese processed below 140°F. is likely to undergo gassy fermentation, that the body of the pro-

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cessed cheese is practically constant when made within the range of 140 to 150°F., and that above 150°F. the body increases rapidly. Further, when the cheese is held in the kettle at 145 to 155°F. there is an increase in the body which is almost proportional to the holding time. Color changes have been noted in cheese processed at higher temperatures (1, 2).

In view of the above observations, the processed cheese in the experiments reported here was made by heating uniformly to 148–150°F., and when this temperature had been reached, drawing the samples from the kettle without delay. All factors were kept as uniform as possible in each case, varying only the particular factor under consideration.

The body of the experimental processed cheese was measured by a mechanical device which determined the crushing strength of an inch cube of the cheese with the force applied being increased at a uniform rate until the inch cube had been crushed to a thickness of half an inch. The results are reported in grams of weight, but since this weight was applied with a mechanical advantage of five, the figures given must be multiplied by five to obtain the actual crushing strength. To minimize the effect of individual differences between cubes, six to ten measurements were made in each case with the cubes cut from duplicate or triplicate samples of cheese. The figures reported are the averages of such measurements. This method of measuring the body eliminates the personal factor and gives results that are easily recorded; the experience with this method indicates that it is more reliable than ordinary personal judgment.

Since texture concerns itself with the finer structure of the cheese for which no physical measurements can be given, observations on this property are recorded in descriptive terms. In this connection the slicing properties as measured on a commercial meat slicer are helpful; the appearance of the cut surface, the thinnest slice that can be cut without breaking, and the tendency of the cheese to cling to the revolving blade, all are indicative of texture.

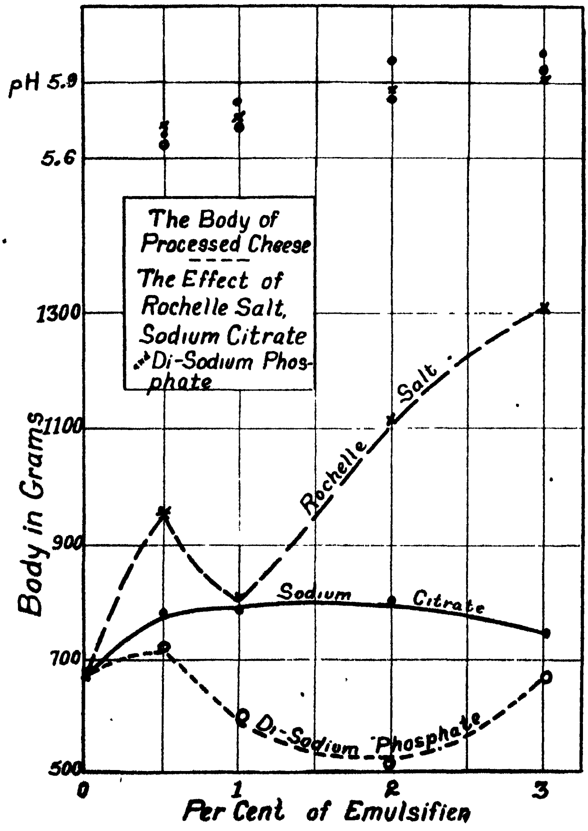
While the main purpose was to study the body and texture, observations were made on,—

(1) The reaction, both pH and titratable acidity,
(2) The appearance of the tinfoil in which the cheese was enclosed,
(3) The color,
(4) The moisture content, and
(5) the keeping quality.

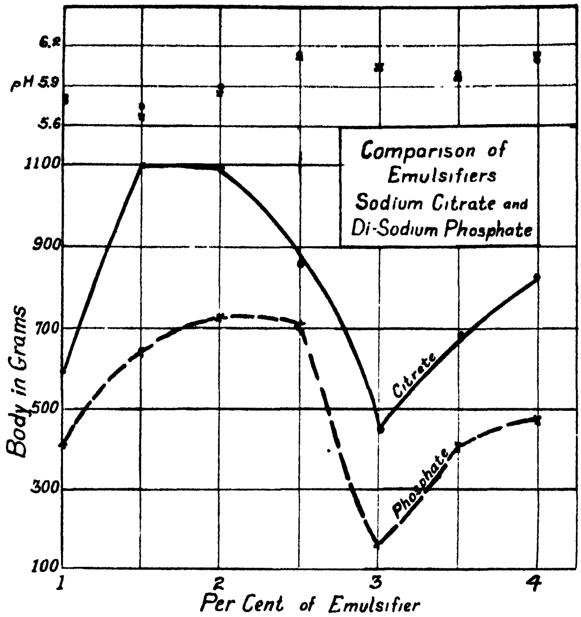
EXPERIMENTAL RESULTS

There are three salts that are commonly used as the so-called emulsifying agents. These are sodium citrate, di-sodium phosphate and Rochelle salt. No explanation will be attempted of the chemical action of these salts.

They are added to prevent fat separation in processing and to control the body and texture. Even when these salts are used, fat separation may occur in the early stages of heating, but in cases where the finished product will be satisfactory, this free fat must again become an integral part of the mass by the time a temperature of 140°F. has been reached. If the fat separation has been excessive or if the fat is not properly re-incorporated, the finished cheese will have a greasy surface, the texture will tend to be coarse and the body weak. With certain cheese or blends (proper age, acidity, etc.) it is possible to produce satisfactory processed cheese without the use of emulsifying salts, but on a production basis the difficulties of selection are so great, and the supply of cheese that meets this requirement is so restricted that the use of emulsifying salts is a practical necessity. Further, even in cases where emulsifying salts would not be absolutely necessary, their use improves the product. A study of the effect of



GRAPH I



GRAPH II

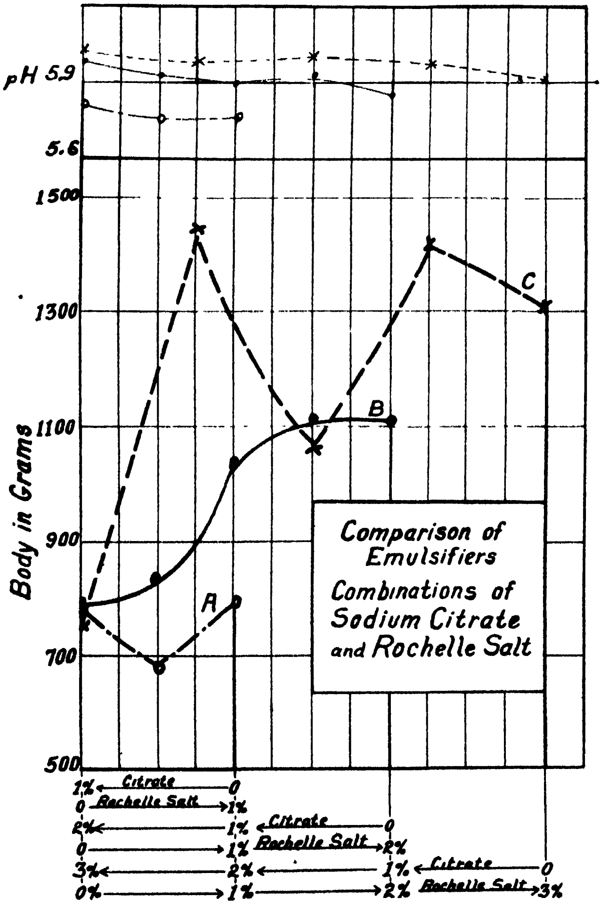
various so-called emulsifying salts in processed cheese is therefore of direct importance.

In accordance with the above, comparisons were made to determine the effect of sodium citrate, di-sodium phosphate and Rochelle salt, individually and in combinations. Graphs I to V inclusive give the results with respect to body.

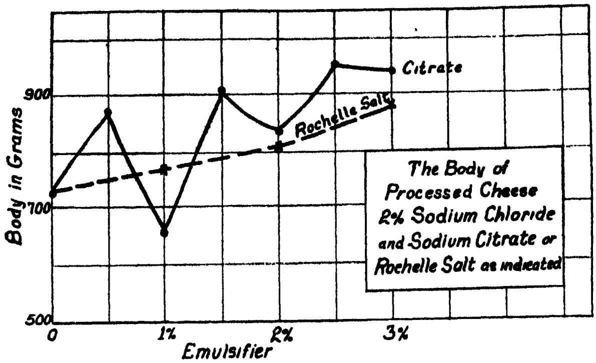
It will be noted in Graph I that Rochelle salt produced the firmest body, and di-sodium phosphate produced the weakest body, with sodium citrate intermediate, at all percentages tried. This confirms the previous report (2) with respect to sodium citrate and di-sodium phosphate, *viz.*, that the former produces cheese of a firmer body than the latter. This is confirmed further by Graph II. Attention is called to the difference in shapes of the curves for citrate and phosphate in Graphs I and II; these variations are due to the fact that different lots of cheese were used. Variations such as these are encountered throughout the experimental work on processed cheese, and so far they have not been related to any specific property of the cheeses involved. This fact must be kept in mind and conclusions can be drawn only where a certain factor shows a general trend quite consistently.

Graph III shows the body of processed cheese when made with various percentages and combinations of sodium citrate and Rochelle salt. Curve

A connects three points representing 1 and 0%, ½ and ½%, and 0 and 1%, sodium citrate and Rochelle salts respectively; Curve B connects five points representing 2 and 0%, 1½ and ½%, 1 and 1%, ½ and 1½%, and 0 and 2% sodium citrate and Rochelle salts respectively; Curve C connects five points representing 3 and 0%, 2.25 and 0.75%, 1.5 and 1.5%, 0.75 and 2.25%, and 0 and 3% sodium citrate and Rochelle salts respectively. In an entirely similar manner Graph IV shows the body of processed cheese when made with various combinations of di-sodium phosphate and Rochelle salts, and Graph V, when made with various combinations of sodium citrate and di-sodium phosphate. Graphs III and IV show that the general trend is an increase in the firmness of the cheese as Rochelle salt replaces sodium citrate and di-sodium phosphate respectively. Graph V, Curves A and B, in general show the expected effect, *viz.*, a decrease in body as phosphate



Graph III



GRAPH VI

gritty particles resembling broken glass were identified as calcium tartrate crystals. In the present experiments no such defect was observed in any of the cheese made with Rochelle salt. Apparently the crystallization of calcium tartrate in cheese occurs only under special conditions which have not been reproduced.

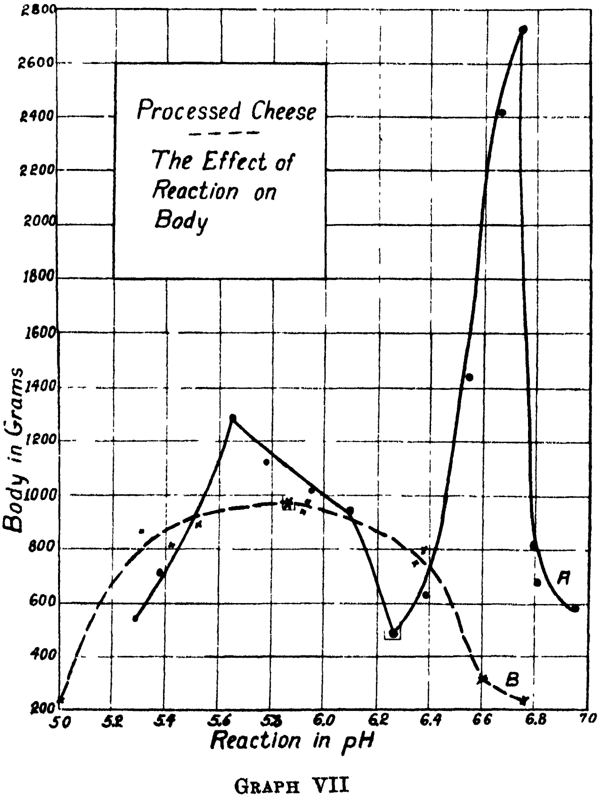
The texture of cheese made with di-sodium phosphate was less satisfactory than either of the above. The cheese was not brittle but soft and soapy. The slicing property is poorer as the cheese tends to cling to the revolving blade. As with Rochelle salt, cracks due to poor fusion were noted. With the larger amounts of phosphate the tinfoil showed discoloration.

From the results of the studies on these salts it was decided to use 2.0% sodium citrate in subsequent experiments where other factors were being studied unless otherwise indicated.

In the manufacture of processed cheese it is quite a common practice to use about 0.5% common salt in addition to that which is already present in the original cheese. Experiments were undertaken to determine the effect of added sodium chloride alone and in combination with emulsifying salts. In experiments comparing the effects of common salt additions up to 2.0%, it was found in general that the body of the cheese decreased with increased salt content, and fat separation during processing and graininess in the texture of the finished cheese increased. Salt additions in excess of one per cent were distinctly noticeable to the taste. In an experiment where 2.0% common salt and sodium citrate or Rochelle salt were added up to 3%, it was found that these emulsifying salts remedied the defects occasioned by the common salt, and there was a general trend of increased body with increases in the amount of emulsifying salt (Graph VI). The finding that the body of the cheese samples containing Rochelle salt was weaker in most cases than corresponding samples made with sodium citrate was unexpected. The only explanation that can at present be suggested is that Rochelle salt was less efficient in overcoming the weakening effect of the salt on the body.

The reaction of the original cheese and of the finished processed cheese is of importance in a number of respects. In commercial processing of cheddar cheese, it is known that the acidity of the cheese used in making the blend cannot be ignored; in this case, however, there is involved not only (1) the direct effect of reaction on the behavior in processing, but also (2) differences in flavor and physical properties resulting from the effect reaction has on the curing processes in the original cheese. The experimental work reported here has concerned itself only with the former of these two points. The direct effect of reaction at the time of processing is important because of its relation to the flavor, keeping quality and physical properties of the finished product, and to the discoloration of the tinfoil, as shown in the previous paper (2).

In order to determine the effect of reaction at the time of processing, experiments were conducted in which a number of samples were prepared



GRAPH VII

from the same lot of original cheese, processed with 2% sodium citrate and with additions of sodium bicarbonate or hydrochloric acid to produce the desired range in reaction. Curve A in Graph VII reproduces the results of such an experiment reported in a previous paper (2); Curve B gives additional results with a different lot of cheese. In the former the cheese was six months old, while in the latter the cheese was over ten months old at the time of processing; there were undoubtedly other differences in the two lots of cheese, so that an attempt to relate the difference in the curves definitely to the age of the cheese is not justified. The boxed point on each curve represents the reaction of the control sample, *i.e.*, the processed cheese made with 2% sodium citrate, but without either sodium bicarbonate or hydrochloric acid; the reaction of these control samples give further indication of a difference between the two lots of cheese.

The results in Graph VII show that the body of the processed cheese may be influenced to a marked extent by the reaction. Curve A differs from Curve B mainly in that the former shows a pronounced increase in body on the alkaline side of the control sample. Disregarding this difference, the curves show a general similarity in that the maximum body comes within the range of pH 5.6 to 6.1.

The texture of the cheese was found to vary with the reaction. Between the limits of pH 5.7 to 6.3 there was little difference in texture; at greater acidity, the cheese had a grainy appearance, was very brittle, and had an oily surface. At the most acid reaction indicated, the cheese was so soft that body measurements were impossible. With decreasing acidity beyond pH 6.4, the texture became more grainy, the surface of the sample was soapy, and the fat tended to accumulate at the upper surface of the sample. The layer below the fat was very coarse grained as compared with the fine granulations in the sample on the acid side of the above limits in which there was no evidence of fat rising.

A pronounced darkening of the tinfoil was observed in the samples of low acidity. In the most severe cases the darkening penetrated into the cheese to a depth of about a fourth of an inch. The tinfoil used in lining the sample boxes throughout this work was of the same grade as is used commercially.

The moisture content of the cheese is known to affect the body and texture, and was therefore kept as uniform as possible in all the experiments. Results previously reported show that when other factors were kept constant the body of processed cheese varied inversely with the moisture content; with high moisture the body was weak and the texture was somewhat grainy with a tendency for fat separation, while at the other extreme the cheese is very firm, dry and brittle. Numerous instances have been found to show that the amount of moisture present in two samples of cheese may be the same and yet the body will be very different. The most striking example of this appears in Graph VII in which two successive points (Curve A) differ in body by almost 2,000 grams.

Reference was made to the age of the two lots of cheese used in Graph VII, and while other factors may well be involved in this case, the somewhat weakér body in Curve B is expected on the basis of age. This becomes more apparent from results previously reported (2) where three separate lots of cheese were processed at different ages ranging from 1 day to 9 months. With one exception in the case of cheese three days old, the body of the processed cheese decreased with the age of the cheese from which it was made. The blending of cheese in commercial practice reduces the variations due to age; usually two or three parts of young cheese (1 to 3 months old) to one part of older cheese (8 to 12 months) are used, the proportions being varied according to the flavor desired.

The body of original cheese becomes weaker with increases in the fat content, the moisture content being held constant. This general observation has been verified by Sammis and Germain (5) who found further that the quality of such cheese decreased with increases of the fat content. Some of the cheese which had been made by the above investigators in their experiments, was used in processing experiments in which the object was to compare two commerical forms of sodium citrate (U. S. P. VIII or 2 $Na_3C_6H_5O_7.11H_2O$ and U. S. P. X. or $Na_3C_6H_5O_7.2H_2O$) and di-sodium phosphate. No appreciable difference was found between the two forms of citrate; the results have been averaged and are given in comparison with di-sodium phosphate in Graph II. The same lot of cheese was not used throughout the experimental work shown in Graph II, but at each percentage of emulsifier studied the cheese used for the phosphate and citrate samples were comparable. Graph II is therefore a fair comparison of the two emulsifiers, but is not to be used to indicate the effect of increasing percentages. For example, the sudden drop in the body at 3% emulsifier was due to the fact that the average age of the cheese was about 9 months as compared with 6½ to 7 months for the others.

While the main object of the experimental work involved in Graph II was as already stated, it was found that when the results were tabulated there was a difference in the body of the processed cheese samples made from each pair of original cheese. When the findings were checked against the more complete record of Sammis and Germain, it was found (as is shown in Table 1) that in each pair of cheeses, the one with the lowest ratio of casein-to-fat produced the weakest body in the processed cheese. This fact that a high fat content weakens the body of processed cheese has been further verified in a study of cheese spreads where it was found that the moisture content of the spreads could be reduced without losing the spreading characteristic if the fat content was increased.

TABLE 1

The relationship between the casein-to-fat ratio in the milk and the body of the resulting processed cheese using different emulsifying salts

CHEESE NO.	UNPROCESSED CHEESE		MILK	PROCESSED CHEESE			
					Body in grams		
					Sodium citrate		Di-sodium Phosphate
	Per cent moisture	Per cent fat in dry substance	Casein-to-fat ratio	Per cent Emulsifier used	U.S.P. VIII	U.S.P. X	
7	*38.0	*53.70	*0.681	2.0	1111	1056	
8	37.4	53.2	0.667	2.0	708	954	
13	38.7	52.8	0.699	3.0	1251	1311	
14	38.2	55.3	0.567	3.0	945	847	
19	35.8	50.46	0.703	2.0	1700		1048
20	34.8	55.2	0.606	2.0	1154		537
21	36.0	52.03	0.714	2.0	1210		712
22	35.6	53.63	0.651	2.0	1007		656
23	37.8	53.54	0.698	1.0	1566	1462	
24	37.2	55.89	0.601	1.0	983	1077	
29	35.2	50.00	0.716	1.0	775		636
30	36.6	56.8	0.544	1.0	543		287
31	39.2	48.65	0.796	1.5	1252		456
32	37.6	59.15	0.559	1.5	342		162
43	39.3	48.80	0.791	1.5		1506	902
44	42.3	56.00	0.546	1.0		832	422
105	40.1	51.08	0.733	3.0	465		208
106	39.7	59.7	0.430	3.0		210	**

* These figures taken from data presented by Sammis and Germaine (5).

** Cheese was too soft to measure.

SUMMARY

From the experimental work described in this paper, a number of conclusions may be drawn:

1. The body and texture of processed cheese are improved by the addition of the salts used as emulsifiers.

2. Sodium citrate gave the most desirable body and texture, Rochelle salt and di-sodium phosphate following in the order named. Defects noted with Rochelle salt were mealiness tending toward graininess and poor fusion of the warm cheese in the molds. Di-sodium phosphate gave a very soft, soapy product that also showed poor fusion.

3. Graininess is associated with lack of air cells in the cheese and is often accompanied by fat separation.

4. The use of one-half of one per cent of sodium chloride is sufficient. More than one per cent is noticeable to the taste and weakens the body of the cheese. The use of the emulsifying agents lessens the body defects caused by the use of sodium chloride.

5. Temperature control is necessary.

6. The reaction of the cheese is important, commercially it is modified by blending. The addition of one per cent of acid or its equivalent of alkali weakens the body of the cheese and produces decided changes in the texture.

7. Between reactions of pH 5.7 and 6.3 the differences in the body and texture are very slight. On either side of this range of reaction defects are noted that become more serious as the difference between the reaction of the sample and the limits set above increases.

8. When all other factors are kept constant the body of processed cheese varies in an inverse ratio with the amount of water that is incorporated in it.

9. The body of the cheese becomes weaker as the age of the cheese used increases. An average age of five to seven months in blending mixtures should give satisfactory results.

10. The casein-to-fat ratio in the milk used for the original cheese has a marked effect upon the body of the processed cheese, the greater the proportion of fat the weaker the body.

In conclusion the authors wish to express their thanks to Chas. Pfizer and Co., Inc., who have sponsored the fellowship under which this work has been done.

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THE SOLUBILITY OF METALS IN MILK:—II. SUBMERGED CORROSION TESTS OF VARIOUS METALS IN MILK[1]

H. T. GEBHARDT AND H. H. SOMMER

The solubility of metals in milk and other dairy products is of interest both from the standpoint of the durability of the equipment, and of the effect of the dissolved metals on the flavor, keeping quality and healthfulness of the products. Formerly the problem was considered as being primarily of interest to the manufacturer of dairy equipment, but with the keener appreciation of the possible effects of the dissolved metals on the dairy products in recent years, the problem has now become of direct interest to the manufacturer of dairy products.

This new view-point of the problem has stimulated research work, but in some respects the knowledge bearing on this problem is still incomplete. Further, the experimental results that have been reported are not quite comparable and their value is conditioned because of inadequate control over such factors as agitation, temperature and composition of the milk especially with respect to dissolved oxygen, as was pointed out specifically for copper in a previous paper (9).

In a detailed study of the corrosion of copper by milk (9) an experimental procedure was developed with the aim of controlling various factors. This procedure has been applied to other metals with special attention to those factors which had been found important in the corrosion of copper. The results of this study are given in this paper.

The various metals studied were in the form of test pieces, 1 by 3 inches but varying slightly in thickness. The test pieces were clamped in a vertical position between the holding arms of the stirring device which revolved at 210 R. P. M. in about 500 cc. of milk. (For further details see previous paper (9).) In the present study the corrosion was determined exclusively by weighing the test pieces before and after exposure. The losses in weight were finally expressed in terms of milligrams per square decimeter of metal surface per hour (mg./dm.^{2}/hr.). Since the same test pieces of the various metals were used throughout, it was possible to compare their relative corrosion resistance not only within each experiment, but also throughout the entire study.

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In order to simulate well cleaned metal surfaces in practice, the test pieces were lightly scoured with Bon Ami powder just enough to remove any tarnish before each exposure. An exception from this general procedure was made in the tests to determine the effect of steam sterilization; in these tests the pieces were scoured, exposed to steam sterilization and then exposed to the milk with whatever tarnish had developed. In these tests the type of tarnish developed by the steam sterilization, and in all tests the type of tarnish resulting from exposure to the milk, was carefully observed.

Using this general procedure, observations are reported as follows in the order given:—(1) the effect of acidity, (2) the effect of dissolved oxygen, (3) the effect of temperature, (4) the effect of steam sterilization, (5) cumulative corrosion losses with intermittent exposure, and (6) the type of tarnish developed.

Table I gives the test pieces that were used, their surface areas, their general source and remarks.

TABLE 1

The test pieces used in the corrosion tests

METAL	SURFACE AREA, DM.^{2}	SOURCE	REMARKS
Aluminum	0.414	U. W. Service Shop	Commercial sheet
Nickel	0.399	Cherry-Burrell Corp.	Commercial sheet
Zinc	0.396	U. W. Service Shop	Commercial sheet
Tinned Copper	0.402	Cherry-Burrell Corp.	Edges tinned
Tinned Copper	0.402	Cherry-Burrell Corp.	Edges soldered
Soldered Copper	0.398	U. W. Service Shop	Solder (50–50) coated
Tinned Iron	0.395	U. W. Service Shop	Edges tinned
Galvanized Iron	0.395	U. W. Service Shop	Edges bare iron
Allegheny Steel	0.399	Cherry-Burrell Corp.	Commercial sheet
Stainless Steel	0.403	U. W. Service Shop	Sheet, one side polished
Monel Metal	0.396	Cherry-Burrell Corp.	Commercial sheet
Brass	0.412	U. W. Service Shop	Sheet, alpha-brass
German Silver	0.405	U. W. Service Shop	Sheet

EXPERIMENTAL RESULTS

It has been quite generally assumed in various investigations on the corrosion of metals that the acidity is an important factor. The effect of acidity on corrosion was therefore given first consideration; the experimental results obtained in the study of this factor are given in table 2 and Graph I.

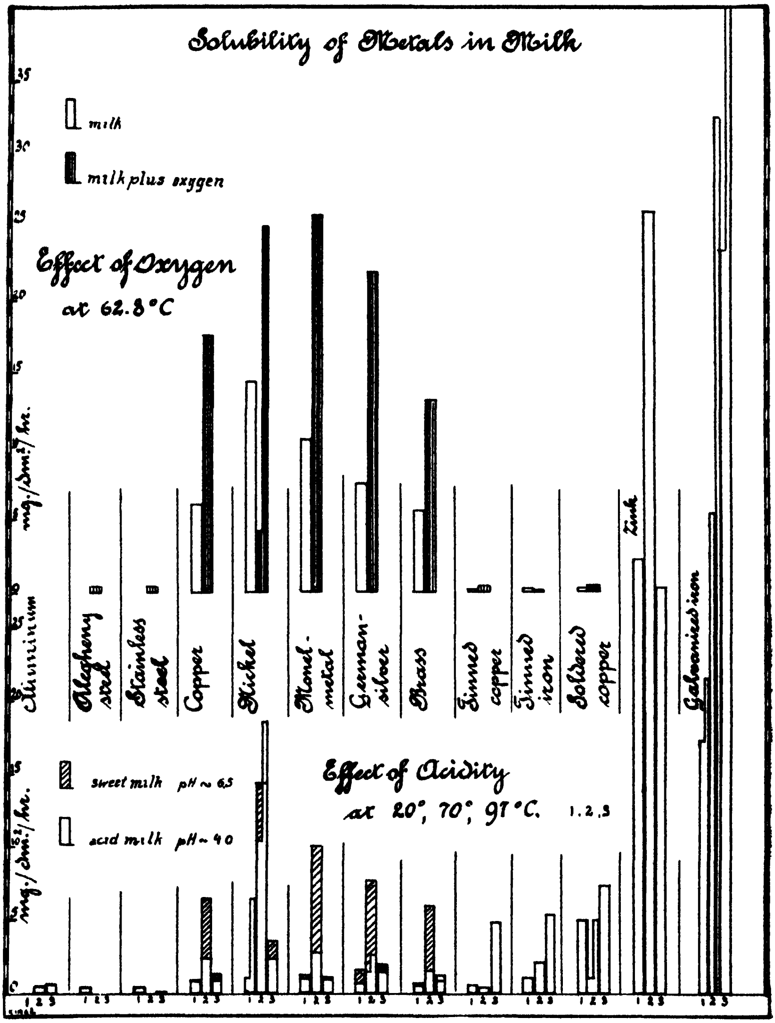
It will be noted that in many cases corrosion increased with acidity, but there are quite a number of cases where this effect is comparatively slight and others where there was a distinct decrease in corrosion with increased acidity. The metals where corrosion increased with acidity at all three temperatures studied, were:—zinc, galvanized iron, tinned iron, tinned copper (edges tinned), solder coated copper, and aluminum (except at 20° C. where there was no perceptible corrosion). Tinned copper (edges soldered) could be added to this group except for the slight decrease of corrosion in high acid milk at 70° C. The group of copper and copper alloys, *viz.*, copper, Monel metal, brass, and German silver, showed less corrosion in high acid milk than in sweet milk in all cases, excepting slightly increased corrosion with increased acidity at 97° C. in the case of copper, Monel metal, and brass. Allegheny steel and stainless steel showed greater corrosion in acid milk at 20° C., no perceptible corrosion in either low or high acid milk at 70° C., and a very slight decrease with increased acidity at 97° C. In the case of nickel, increased acidity caused an increase in corrosion at 20° C., variable results at 70° C., and a decrease at 97° C.

The variable results in the case of nickel as mentioned above are typical for this metal. Greater variations in duplicate or parallel experiments were encountered in the case of nickel than with any other metal studied. This was apparently occasioned by differences in the condition of the metal surface, but even with special care in cleaning or scouring the nickel, wide variations occurred in duplicate tests.

It should be noted here (referring to Footnote (1), table 2) that corrosion was greatly increased by connecting the test piece to a more noble metal (platinum electrode). In the case of aluminum the corrosion under such conditions was strictly localized in the form of numerous pin-point pits. At these points particles of milk solids adhered very tenaciously.

The Effect of Oxygen

The rôle of oxygen in copper solution has been recognized for some time. The results given in the previous paper (9) have demonstrated pronounced increases in the amount of copper dissolved in milk in the presence of oxygen as compared with conditions where less oxygen is present. In view of these results, the effect of oxygen on the corrosion of the various metals under consideration, was studied. Oxygen was bubbled through the milk during the entire period of exposure of the metal test pieces to the milk. The vessel containing the milk was closed except for a small gas vent, so that the atmosphere above the milk was substantially pure oxygen at atmospheric pressure. The losses in weight of the test pieces were compared with similar tests where the milk was in equilibrium with air. It would have been desirable to have studied this factor at various temperatures, but time limitations restricted it to one temperature. A temperature of 62.8° C. was chosen because it represents the usual pasteurizing temperature and because this temperature is in the vicinity of the temperature of maximum solubility for most metals. The results are given in table 3 and Graph I.



GRAPH I

It will be noted that in the presence of an increased oxygen content the corrosion of all the metals increased with the exceptions of aluminum, which showed no perceptible corrosion in either case, and of tinned iron and stainless steel which showed decreased corrosion in the presence of more oxygen. Further comment on this factor will be made later in the discussion.

Temperature

In the previous paper (9) it had been found that the solubility of copper increased markedly with the temperature up to a maximum of

TABLE 2

The effect of acidity on the solubility of metals in milk

METAL	*(Mg. loss in weight per sq. dm. of metal surface per hr.)* 20°C. pH 4.03*	20°C. pH 6.6	70°C. pH 4.00*	70°C. pH 6.6	97°C. pH 3.85**	97°C. pH 6.6
Aluminum	0.0	0.0	2.4(1)	0.0	0.67	0.0
Copper	0.82	0.90	2.40	6.45	1.82	1.34
Nickel	6.47 1.06	0.69	18.60 10.40	14.3	2.36	3.60
Zinc	27.2	2.38	51.0	1.59	25.2	8.66
Tinned Copper (Edges Tinned)	0.60	0.10	0.40	0.20	4.68	0.10
Tinned Copper (Edges soldered)	0.37	0.10	0.16	0.20	0.17	0.27
Solder coated Copper	4.92	2.79	1.06 4.90	0.91	7.03	0.95
Tinned Iron	1.01	0.38	2.20	0.33	5.38	3.52
Galvanized Iron	19.12 14.74	6.31	30.3(2) 57.5	1.87	48.3(2) 125.2	1.77(3) +1.52
Allegheny Steel	0.40	0.0	0.0	0.0	0.0	0.05
Stainless Steel	0.42	0.0	0.0	0.0	0.0	
Monel Metal	1.0	1.23	2.8	10.90	1.18	0.91
Brass	0.0 0.68	1.50	1.60	6.00	1.21	0.92
German Silver	0.65	1.67	2.70(1) 1.40	7.75	1.13	2.00

* Whole milk ripened with a starter to maximum acidity.

** Whey ripened to maximum acidity.

(1) Metal test piece was connected to a platinum electrode.

(2) The greater losses were observed with older test pieces which had already had suffered corrosion.

(3) The old test pieces gained in weight, while the new one suffered losses.

70° C., beyond which the solubility sharply declined, so that it was about the same at boiling temperature as at room temperature. This point of maximum solubility differed appreciably from previous findings, and the difference was attributed to the lack of agitation in the work of Rice and Miscall (28) and unusual opportunities for oxygen solution in the work of Quam (26).

The effect of temperature on the corrosion of other metals has been studied by several investigators but by methods concerning which similar questions may be raised. In the present study of the effect of temperature, agitation was provided by the procedure already described. The results obtained are given in table 4 and Graph II.

It will be noted that the temperature of maximum solubility is at 70° C. for copper and copper alloys, *viz.*, copper, brass, Monel metal and German

TABLE 3

The effect of oxygen on the solubility of metals in milk

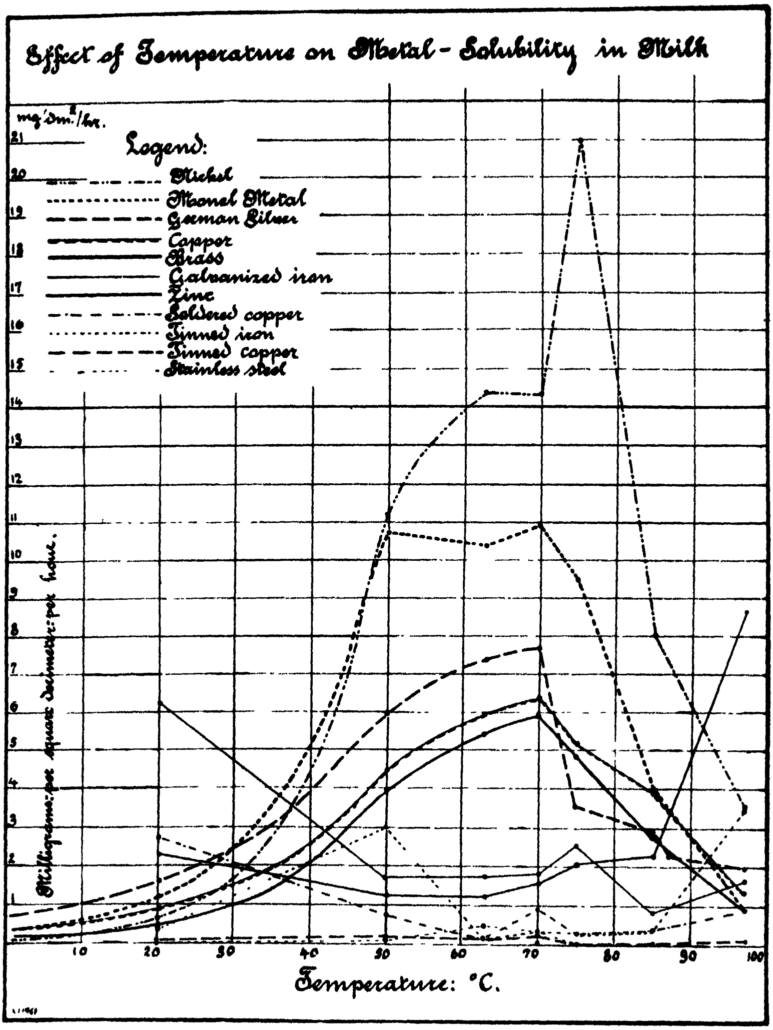
METAL	(mg. loss/sq. dm./hr.)	
	CONTROL 62.8°C.	OXYGEN 62.8°C.
Aluminum	0.0	0.0
Copper	6.00	17.65
Nickel	14.40	4.13 25.10
Zinc	1.26	2.87
Tinned Copper (Edges Tinned)	0.20	0.35
Tinned Copper (Edges Soldered)	0.10	0.15
Solder Coated Copper	0.20	0.38
Tinned Iron	0.18	0.00
Galvanized Iron	1.78	5.79
Allegheny Steel	0.10	0.45
Stainless Steel	0.52	0.27
Monel Metal	10.47	25.80
Brass	5.50	13.17
German Silver	7.41	21.90

silver, with a marked decrease in solubility on either side of this temperature. The same holds true for nickel except that the maximum is at 75° C. The solubility was so low and varied so little with temperature in the case on aluminum, Allegheny steel (not shown on Graph II), stainless steel and tinned copper that no definite conclusion can be drawn as to the tempera-

TABLE 4

The effect of temperature on solubility of metals in milk

METAL	(mg. loss per sq. dm. of surface per hour)						
	20°C.	50°C.	62.8°C.	70°C.	75°C.	85°C.	97°C.
Aluminum	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Copper	0.90	4.54	6.00	6.45	5.24	4.00	1.34
Nickel	0.69	11.20	14.40	14.30	21.00	8.00	3.60
Zinc	2.38	1.26	1.26	1.59	1.07	1.24	8.66
Tinned Copper (Edges Tinned)	0.10	0.20	0.20	0.20	0.00	0.00	0.10
Tinned Copper (Edges Soldered)	0.10	0.00	0.10	0.20	0.20	0.00	0.27
Solder Coated Copper	2.73	0.72	0.20	0.91	0.25	0.38	0.95
Tinned Iron	0.38	3.06	0.18	0.33	0.28	0.49	3.52
Galvanized Iron	6.31	1.77	1.78	1.87	2.60	0.82	1.77 +1.52
Allegheny Steel	0.00	0.25	0.10	0.00	0.03	0.00	0.05
Stainless Steel	0.00	0.12	0.52	0.35	0.00	0.00	. ..
Monel Metal	1.23	10.00	10.47	10.90	9.50	4.10	0.91
Brass	1.50	4.00	5.50	6.00	4.90	2.80	0.92
German Silver	1.67	6.05	7.41	7.75	3.65	2.96	2.00



GRAPH II

ture effect. In the case of zinc, galvanized iron, and solder coated copper there is a well defined tendency to show a minimum solubility in the temperature range where copper, copper alloys and nickel showed maximum solubility.

The Effect of Steam Sterilization of Metal on Corrosion

The oxidation of the metal surface can be expected to influence metal solution. Rice and Miscall (28) have reported immense increases in the amount of copper dissolved from oxidized surfaces as compared with bright copper. Copper oxidized by holding it momentarily in a Bunsen flame, dissolved 3.5 times as much, and copper oxidized by immersion in chlorinated soda solution dissolved 5 times as much as bright copper.

Experiments of this nature are of interest, because in practice the sterilization of equipment affords opportunity for oxidation of the metal surface. In a study of this factor steam sterilization was chosen because it is most common in practice, and by the use of an autoclave the treatment could be made quite uniform. The metal test pieces were laid across glass rods so that they were not in contact with each other or with autoclave wall. The test pieces were sterilized for 15 minutes at 15 pounds' steam pressure in an atmosphere of steam which undoubtedly contained some air. The test pieces then subjected to the corrosion test without future treatment. The results are given in table 5. Steam sterilization of the metal caused pronounced increases in the corrosion of zinc and galvanized iron, a slight increase in the case of copper, a slight decrease in the case of brass, and pronounced decreases in the case of Monel metal and nickel.

Cumulative Corrosion Losses

The same metal test pieces were used in the various experiments in this study in order to enable a comparison of the cumulative weight losses as the result of various exposures to milk followed each time by light scouring. The results of such a comparison are given in table 6 with the metals arranged in order of their weight losses.

The Tarnishing of the Metals

The metal test pieces were examined after each exposure to observe tarnishing. Of the metals studied only nickel and Monel metal showed pronounced tarnishing in the submerged corrosion tests. The degree of tarnishing varied with the temperature; it was most intense where the rate of solution was highest. Nickel developed a gray tarnish at 50° C. which was more pronounced at 62.8 to 70° C., and at 97° C. At 75° C. a bluish tarnish developed which was darkest near the edges, and yellowish near the center. At 85° C. the tarnish was distinctly brown, darkest near the edges, indicating stream lines. In acid milk at 20° C. it was brown gray, showing fern-like designs, at 70° C. it was gray and at 97° C. practically no tarnish developed.

Monel metal became dull at 20° C.; at 62.8° C. tarnishing was more intense, bluish gray at the edges with reddish brown at the center of the test pieces; at 70° C. the tarnishing was similar but even more intense. At still higher temperatures the tarnishing was less pronounced. In acid milk at 20° C. practically no tarnish developed; at 70° C. the surface became slightly gray; and at 97° C. it was dark brown near the edges.

Treatment of the metal in the autoclave produced brown-red tarnishing of copper and brass; nickel became dull with a slight gray-brown tarnish;

zinc and galvanized iron became coated with white zinc oxide powder. The appearance of the other metals was not influenced to any extent by steam sterilization.

DISCUSSION OF EXPERIMENTAL RESULTS

The Effect of Acidity on the Corrosion of Metals by Milk

The acidity has been generally assumed to be the most important factor in metal corrosion; most of the corrosion tests reported in the chemical engineering literature were made with acid solutions as the corrosive medium. This idea was carried over to the study of the corrosive action of milk on metals. Thus, Donauer (2) who made the first study of the relative corrosion of metals by milk, in planning his experiments was guided by the idea of the prime importance of acid in metal solution. He compared the action of milk and partly soured milk with acidities of 0.20 and 0.26 per cent (calculated as lactic acid) with that of a 0.20 per cent lactic acid solution. He concluded that the corrosion was much higher in ripened milk than in sweet milk but less than in the lactic acid solution. Seligman (30) also suggested the importance of the acidic constituents of milk. Rice and Miscall (29) concluded:—"Milk slightly sour dissolves but little more copper than normal milk." North (25) studied the behavior of metals in beaker tests, in solutions of lactic acid and hydrochloric acid of various concentrations, apparently to bring out the relative corrosion resistance of various metals to be used in dairy equipment. Hunziker, *et al.* (14), concluded:—"In general the corrosive effect on the

TABLE 5

The solubility of metals as affected by steam sterilization

METAL	AMOUNT OF METAL DISSOLVED	
	STEAM-STERILIZED Loss in mg. /dm.^{2}/hr.	UNTREATED Loss in mg. /dm.^{2}/hr.
Aluminum	0.0	0.0
Allegheny Steel	0.0	0.0
Stainless Steel	0.0	0.0
Tinned Copper, Edges Tinned	0.0	0.0
Tinned Copper, Edges Soldered	0.0	0.0
Tinned Iron, Edges Tinned	0.0	0.5
Monel Metal	0.0	10.5
Nickel	9.9	14.4
Copper	6.3	6.0
Brass	5.1	5.5
Galvanized Iron	10.4	1.8
Zinc	30.1	1.3

The metals were agitated in milk at 62.8° C. during one hour.

The values given are the averages of four determinations; with the exception of brass, where it is the average of three.

TABLE 6

Cumulative losses of the various metal test pieces

METAL	NUMBER OF EXPOSURES	TOTAL LOSS PER SQ. DM.	LOSS PER SQ. DM. PER EXPOSURE
		mg.	*mg.*
Aluminum	12	0.85	0.07
Allegheny Steel	11	1.28	0.12
Stainless Steel	8	2.69	0.33
Tinned Copper (Edges Tinned)	12	16.48	1.37
Tinned Copper (Edges Soldered)	10	24.60	2.46
Tinned Iron	12	42.15	3.52
Zinc	10	41.10	4.11
German Silver	10	43.20	4.32
Brass	10	63.70	6.37
Copper	10	64.70	6.47
Monel Metal	10	114.60	11.46
Galvanized Iron	9	111.70	12.40
Nickel	9	113.50	12.60
Solder Coated Copper	8	196.00	24.50

These cumulative losses represent the total loss in weight after the number of tests or exposures indicated, followed after each test by cleaning with Bon Ami powder. These figures are intended only to give a general indication of the relative durability of the metals.

metals was greater in acid solutions than in the milk products. This was evident even where the acidity of the milk product was equal to or greater than that of the acid solution. This suggests that the acidity is the chief factor in metallic corrosion and that some of the non-acidic milk constituents apparently exert a corrosion protective action. With minor exceptions, the corrosion was more intense in the high acid products than in the low acid products, and at high temperatures than at room temperature. But even in sweet milk products and at room temperature corrosion was by no means absent.''

On the other hand, Frazer *et al.* (8) in a study of the solubility of Monel Metal in sulphuric acid solutions found a constant decrease of the solubility with increasing hydrogen-ion concentration, in solutions from 0 to 80 per cent (by weight) sulphuric acid.

From the experimental results reported here (table 2) it appears that the question of the rôle of acidity in metal corrosion must be answered individually for each metal and for various temperatures. As has already been pointed out in connection with the presentation of table 2, a number of instances were found where increased acidity actually cause decreased corrosion. Further, a comparison of the quantitative data (tables 2, 3 and 4) leads to the conclusion that the effect of acidity is never as great as that of dissolved gases and temperature. Acidity, therefore, can not be considered as the chief factor in metallic corrosion in milk.

The Effect of Dissolved Gases on the Corrosive Action of Milk

The increased corrosion and pitting of metals at the liquid-air-line has been mentioned by investigators (11) as being indicative of the importance of oxygen in metal corrosion. However, it is not correct to draw conclusions concerning the effect of dissolved oxygen on metal solution from such partial immersion, beaker tests, because, as has been shown by Evans (4) and others, in such tests electrochemical phenomena appear, which offset the mechanism of ordinary metal solution. In such tests differential aeration cells are set up which determine the rate and the location of metal corrosion entirely by an electrochemical mechanism.

While the rôle of oxygen in copper solution has been studied and has been appreciated for some time, this is not the case with other metals. It is rather recent that this factor has received attention in the chemical engineeding literature on corrosion of other metals.

The results reported in the present paper show that oxygen plays an important rôle in the solution of all metals. An increased oxygen content increased metal solution, with few exceptions (aluminum and tinned iron).

The study of the effect of dissolved oxygen is somewhat difficult and not absolutely conclusive, because a relatively long time is required to obtain a point of saturation, or a point of equilibrium with respect to dissolved gases. Whether such a point has been reached cannot readily be determined. Another complication in such a study is the possibility that an excess of oxygen may cause the formation of a protective film which prevents further metal solution; conversely the removal of oxygen may lead to the solution of protective films and thus cause increased corrosion.

The Effect of Temperature on the Corrosive Action of Milk

Various studies on the effect of temperature on metal solubility in milk have been reported (2, 28, 25, 26, 27, 14, 24); the temperature-solubility relationship has been studied most extensively for copper. Extensive data on copper have also been reported in a previous paper (9). Most of the work on metals other than copper (except that by Quam) has been based upon beaker tests which (because of lack of agitation) must be expected to give results that are not comparable with practical conditions or with the results reported here.

Quam has studied the solubility of nickel, tin, chromium steel, aluminum, zinc, and copper in milk at various temperatures. He found the solubility of nickel to be at a maximum at 75° C. and about equal at 50° and 95° C. For copper and zinc he found solubility that were nearly alike, and he found the temperature of maximum solubility for zinc at 75° and for copper at 90° C.

In the present study somewhat different results were obtained. The temperature-solubility curves for the copper alloys were found to be very similar to the curve for copper as previously reported (9). The coincidence of the temperatures of maximum solubility of copper and copper alloys appears to be more than accidental, and seems to indicate an identity of factors involved in their solution, *viz.*, oxygen or oxidation-reduction potential and temperature (9). The fact that the temperature of maximum solubility for nickel is approximately the same (75° C.) indicates that its solution is also affected by the same factors.

Zinc dissolved to the greatest extent at 97° C. and at room temperature, with distinctly less being dissolved at intermediate temperatures; the minimum solubility was found at 75° C. This finding is directly contrary to that reported by Quam. A similar tendency to approach a minimum solubility in the vicinity of 70° C. was observed in the case of galvanized iron and solder coated copper. This tendency to approach a minimum at the temperature where copper and copper alloys show a maximum solubility indicates that the oxidizing conditions which prevail at that temperature confer protection against solution in the case of zinc, galvanized iron and solder coated copper, rather than aid solution as in the case of copper and copper alloys.

It is of interest to note in table 2 that old test pieces of galvanized iron gained weight at 97° C. in sweet milk, while new test pieces lost weight, but lost much less than zinc test pieces under similar conditions. The latter observation must be attributed to the mutual protection resulting from the combination of zinc and iron.

The Effect of the Condition of the Metal Surface

In the experimental work the metal test pieces were cleaned and scoured in a manner designed to simulate practical conditions. Beyond this no work was undertaken to study the effect of the condition of the metal surface specifically. There is reason to believe that the condition of the metal surface, both from the standpoint of metallographic structure and the presence of protective films, is an important factor in metal solution. Therefore every effort was made in the cleaning and scouring to arrive at the same surface condition in all tests. In spite of these precautions wide differences were found in the corrosion of nickel (see tables 2 and 3) which were undoubtedly due to differences in the condition of the surface. This is at variance with conclusion of Frazer *et al.* (8) who stated that in submerged corrosion the shape and finish of the metal test is of minor importance. Their conclusion, however, was based on the behavior of Monel metal in sulphuric acid solutions during 24 hours' exposure. It is to be expected that in short time exposures (one hour in the present study) the effect of finish is more important than in longer exposures, because the initial rate of metal solution is frequently affected by visible or invisible films of oxide or other compounds, which may gradually dissolve or form during exposures as the case may be.

THE RELATIVE CORROSION RESISTANCE OF METALS

Aluminum

Aluminum proved very resistant to corrosion in milk under most experimental conditions. The observation that it corrodes more rapidly when in contact with other metals is in harmony with previous reports (Seligman (30), Hunziker (13, 14)). The great resistance to the action of sweet milk at various temperatures had also been observed by Quam (26). Aluminum thus ranks as one of the most resistant metals when in contact with milk.

The aluminum used in the present tests was wrought aluminum, which is known to be more resistant than cast aluminum (Seligman (30)). Different results may therefore be expected with cast aluminum.

The merits of aluminum as indicated above are in agreement with reports of the successful use of this metal in the milk industry in Europe. Aluminum has proven especially suitable for holding tanks, milk cans, and small pieces of dairy equipment. Fillinger (6), Utz (32), Drouilly (3), and Faragher (5) have also recognized the value of aluminum in handling dairy products. Knoch (18) states that aluminum is not well suited for holding tanks because it suffers serious pitting when used for that purpose. This is not in agreement with the practical experience of others (Verbands-Molkerei, Zurich) where aluminum tanks have been used for years, and still have the same smooth and bright appearance as if new. The difference in experience may possibly be due to having aluminum in contact with other metals in the former case.

The main difficulty in the use of aluminum equipment is that it is not resistant to corrosion by ordinary alkaline washing solutions. There are, however, suitable cleaning procedures available which avoid this corrosive effect. Another handicap to the use of aluminum is the fact that aluminum cannot be soldered by methods now available. The fact that contact with other metals greatly increases corrosion and pitting of aluminum is a further handicap with equipment of a type where pipe lines, valves and other fittings (of different metals) must be connected.

Dissolved aluminum in dairy products, in the small amounts that may be ingested with dairy products handled in aluminum equipment is not harmful to the consumer, as is evident from the work of various investigators (7, 17, 16, 20, 21).

Copper and Copper Alloys; Monel Metal, German Silver, Brass

Copper and copper alloys can well be discussed as a group, because copper is common to all of them and they have very similar properties with respect to their corrodibility. The solubility of these metals varies in such a manner with different conditions, that unquestionably the effect of the constituents of the alloys becomes evident; for example, a comparison of the tarnishing and corrosion of Monel metal and German silver with nickel at various temperatures, oxygen conditions and acidities, clearly indicates the effect of nickel in the former two metals.

The present experimental results differ from those reported by Hunziker *et al.* (14) with respect to the relative corrosion resistance of the metals in this group. These investigators stated:— "—in considering the availability of these copper alloys (nickel silver and Monel metal) for dairy equipment it should be emphasized that these alloys gave evidence of considerably greater resistance to corrosion than copper. This is especially true for Monel metal and to a lesser extent of Nickel Silver."

In line with their conclusions as quoted, they found that Nickel Silver proved more damaging to the flavor of dairy products than Monel metal, and that the former was tarnished to a greater extent. In the present study German Silver was found appreciably more resistant to corrosion than Monel metal (*e.g.*, see table 6). The difference in results is most probably due to differences in the experimental procedures used, and possibly differences in the alloys used.

Considering the known detrimental effect of copper on dairy products, it is of interest to know what proportion of the dissolved alloys consists of copper. Hunziker *et al.* (14) state,—"it appears that it is usually chiefly the copper that goes into solution." They cite a report of the Sheffield Farms, which indicates that German Silver in dissolving yielded mainly copper. In a similar manner Seligman (30) states that mainly copper passes into solution from German silver or Nickel silver. However, no experimental evidence has been presented to substantiate these conclusions.

To examine the correctness of these statements, the proportion of copper represented in the weight losses of German silver, Monel metal and brass on a one hour exposure to milk, was calculated from the observed weight losses and the increase in copper content of the milk as determined by a method previously reported (10). The results are given in table 7. The results indicate that the percentage of copper in the weight loss varies with the experimental conditions; the highest percentage of copper was found in the weight loss from German silver when exposed to sweet milk at 70° C., while in acid whey at 20° C. copper composed a much smaller fraction of the loss. The percentage of copper in the weight loss in sweet whole milk at 70° C. was in close agreement with the percentage of copper in the alloy; only in the case of German silver at 70° C. in sweet milk was there a distinct tendency towards the solution of more copper. All the copper alloys showed less copper dissolving in the milk per square decimeter of surface than did copper under like conditions.

TABLE 7

The percentage of copper in corrosion losses from copper alloys

METAL	CONDITIONS OF EXPOSURE	MG. OF COPPER IN MILK*			WEIGHT* LOSS OF METAL, MG.	PER CENT COPPER	
		Before exposure	After exposure	Amount dissolved		In corrosion	In alloy
Copper (Control)	Sweet Milk, 70° C.	0.132	8.455	8.323	8.05	103.4	100.0
Brass	" " "	0.132	4.463	4.331	7.05	61.45	67.0
Monel Metal	" " "	0.132	2.356	2.224	7.60	29.3	29.0
	" " "	0.132	5.885	5.753	7.55	76.25	
German Silver	" " 75° C.	0.1426	3.974	3.831	5.90	64.8	48–67
	Acid Whey, 20° C.	0.150	0.317	0.167	1.05	15.9	

* The experiments were conducted in duplicate, in each test two metal test pieces were exposed to 530 cc. of milk (or whey); the figures given represent the weight loss from all four test pieces, and the amount of copper found by analysis in 1.060 cc. of milk (or whey).

On the basis of cumulative weight losses as shown in Table VI the metals rank as follows:—German silver, brass, copper, and Monel metal; the latter was corroded considerably more than the other three.

Nickel

Nickel and Monel metal both showed high corrosion losses, with the former showing the higher loss. The tendency of these two metals to tarnish especially in hot milk also distinguishes them.

Reports in the literature on practical experiences with nickel equipment vary considerably, some stressing the advantages and others the disadvantages of nickel. The experimental data reported here may help to elucidate the causes for these varying experiences. In the past, the rate of nickel solution was related mainly to the acidity of the corrosive medium. No consideration has been given to the effect of oxygen on the rate of nickel solution, and just this factor explains the failures with nickel when used with such equipment as surface coolers, cheese hoops and certain cheese vats (23).

The rapid rate of nickel solution at higher temperatures in milk has been reported by Quam (26) and McKay *et al.* (23). This high corrodibility and intense tarnishing observed at high temperatures is in line with practical experiences with nickel pasteurizing vats. The fact that in certain cases nickel pasteurizing vats have been used with success, may have been due to steam sterilization, or some treatment of the metal surface (polishing) which produced some degree of passivity of the metal. McKay (23) recommended the use of abrasives for cleaning nickel equipment. As has already been pointed out, nickel showed pronounced variations in corrosions tests and the variations were apparently due to differences in the surface condition.

Another factor which may greatly influence the behavior of nickel has been mentioned by Hunziker, *viz.*, the presence of aluminum fittings in all nickel equipment, causing a decrease in the corrosion of the nickel. A protective effect of a similar nature is produced by a cathodic treatment of the nickel surface by a current density of 0.0043 Amp/dm.^{2} as described by McKay *et al.* (23). These effects, however, are not specific for nickel alone.

In comparison with copper, it may be said that nickel is noticeably less resistant to corrosion by milk. The effect of dissolved nickel on milk and other dairy products, however, is different from that of copper, as has been pointed out repeatedly in the literature (30, 13, 14, 23, 22, 12, 18 and 30).

Zinc and Galvanized Iron

In the discussion of the value of zinc and galvanized iron for dairy equipment, the opinions expressed in the literature (1, 2, 14) agree that these metals should be excluded from such use. While dissolved zinc has no known detrimental effect on the keeping quality of dairy products, it does have an immediate effect on their flavor. The low resistance of zinc and galvanized iron towards steam sterilization, and their rapid rate of solution in fresh milk, as shown in the present study, furnish further reason for their exclusion. It may be of interest to note that the corrosion of zinc and galvanized iron (table 6) is no greater than some other metals which have not been so generally excluded from use in dairy equipment.

Tinned Copper, Tinned Iron, Solder Coated Copper

These metals are considered in a group because of their similar behavior in contact with milk. The resistance of tinned copper qualifies this metal to rank among the most suitable for handling milk. Its extensive use in practice confirms its merits. The experimental observations of various investigators on the corrosion resistance of tinned copper, in general, justify this conclusion.

There is, however, some disagreement concerning the corrosion resistance of pure tin in sweet milk. Donauer (2) reported that tin is more soluble than copper; Hunziker *et al.* (14), also reported relatively high losses of tin in sweet milk. On the other hand, Quam (26) found no loss in weight at any of the temperatures used. Rice and Miscall (28) suggest that tin probably dissolves more rapidly when in contact with bare copper, but give no experimental evidence to prove this.

The present findings indicate that the tin coating dissolves but very slightly in sweet milk, and that the tin coating suffers no great attack if the proper cleaning agent is used. The rapid wear of the tin coating as frequently observed in practice and which represents the main drawback of this metal surface, is not due to the action of the milk, but rather to the cleaning methods used. Hunziker *et al.* (14, 15), mention that occasionally it is the poor quality of the tin coating which brings about the rapid wear. They suggest that efforts should be made to produce some improved method of tinning, leading to better contact and greater permanence of the coating.

Solder coated copper is not commonly used, except in isolated cases where vats are "retinned" with solder rather than with pure tin. Solder is quite generally used for repair purposes, and to make smooth joints, fill cracks, etc., and therefore is quite frequently exposed to milk. The fact that solder actually dissolves in sweet milk is of interest, because of the toxic properties of lead. Large surfaces of exposed solder should therefore be avoided, although it is known, that direct lead poisoning does usually not occur through ingestion of small amounts of lead in the food.

Tinned iron constitutes the chief material for construction of milk and cream cans, cheese vats, and various small utensils. Practical experience, showing that the tin coating on the iron is relatively short lived, is in agreement with the experimental results reported here. Dissolved tin in small quantities does not affect the flavor and keeping quality of dirty products; the wearing off of the tin, however, permits solution of the exposed iron which is detrimental to the flavor and keeping quality.

The present experimental data do not explain the accelerated corrosion and pitting of tinned iron as it is often observed in practice, because such corrosion is the result of electrolytic action. The mechanism involved in such corrosion has been well illustrated by Kohman and Sanborn (19) in a study of the protective qualities of tin plate in the cans used by the canning industry.

Allegheny Steel, Stainless Steel

Allegheny steel and stainless steel were found to be more resistant to corrosion than any of the other metals, except aluminum; aluminum was fully as resistant under all conditions except at a high acidity and high temperatures. The corrosion losses of the two steels were so low on one hour exposure that no difference could be designated, but the cumulative corrosion losses (table 6) indicated that stainless steel was corroded about twice as much as Allegheny steel. Other investigators (Hunziker *et al.* (14, 15), Quam (26), MacQuigg (22)), have reached similar conclusions. These metals have proven very satisfactory in practice, and a number of reports of their satisfactory application are cited by Hunziker *et al.* (14).

While these steels showed practically no corrosion under the conditions of the solubility tests, it is known from experimental evidence and practical observations that these metals are vulnerable when in contact with other metals. This may occur in the form of bolts, rivets, fittings, or even impurities in the welding. Profuse corrosion may result in such cases, causing these steels to be no more resistant than ordinary steels.

The setting up of an electrolytic cell has been suggested as an explanation for the change in the behavior of these metals in contact with other metals, and further, that the intensity of the attack varies with the potential of the other metal (Hunziker). No experimental proof to suport this theory has been given. It is quite probable that the setting up of a potential difference alone (as assumed by North) is not sufficient to cause this change in resistance to corrosion. The detrimental effect of scratches in the polished surface and the known importance of highly polished surface emphasize that other factors must be involved in the corrosion mechanism of the chromium-nickel steels.

SUMMARY

1. Information concerning metal solution in milk is of great interest to the dairy manufacturer because of the effect of dissolved metals on the color, odor, flavor, healthfulness and keeping quality of dairy products;

further, such information is needed by the dairy engineer for the proper construction of equipment.

2. The data in the literature on the solution of metals in milk have quite generally been obtained by procedures that did not give sufficient control over important factors, *viz.*, velocity of the liquid to the metal, composition of the milk (especially gaseous content), temperature, and the surface condition of the metal.

3. Data are presented for various metals to show the effect of variations in the acidity, oxygen content and temperature, and of steam sterilization on the corrosion losses.

4. The solution of Allegheny steel, stainless steel, aluminum, and tinned copper is very slight in acid milk at room temperature, and in fresh milk at various temperatures and oxygen contents.

5. On the basis of the data presented the relative corrosion resistance of the various metals is discussed.

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THE DIGESTIBILITY OF PROTEIN OF DRIED MILK MANUFACTURED BY DIFFERENT PROCESSES

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The problem of dried milk is becoming important in human welfare. Studies have been made with the products in many directions. Recently the vitamin contents of dried milk have been considerably studied. The results as reported are, however, not so consistent. The inconsistency is not limited to the results of the vitamin studies only, but it is also true in all other chemical and physical studies of dried milk. The reason for this inconsistency is apparent, as the methods of manufacture are very different with different dried products, which give a considerable change in the properties of milk.

The inconsistency of the chemical and physical properties of dried milks led the American Dried Milk Institute to lay different standards of grading dry skimmilk according to different methods of manufacture. This is rather unfortunate for the unification of the standard, but is perhaps necessary at the present stage of the progress of the industry.

In course of study on the osmotic pressure of reconstituted milk from different makes of dried milk, it was accidentally noticed that the digestibility of protein of dried milk may vary with different processes of manufacture. Since in Japan the dried milk is principally used for infant feeding, it was deemed necessary to study the digestibility of protein of dried milks manufactured by different processes to find the best process of manufacture or way of handling the products which are intended for human nutriment, especially for infant feeding. It is hoped that this may lead to the improvement of the manufacturing process and finally to the betterment of the industry.

PRELIMINARY EXPERIMENT

The dried milk was reconstituted by dissolving it in distilled water with Gilchrist mixer in proportion of 13 grams of powder to 100 cc. water at 40°C. Four typical brands of dried milk sold on Japanese market were selected and used for the experiment. The names of the brands are Kintaro, Klim, Morinaga, and Lactogen. The former two are the dried milk manufactured by spray process and the latter two are by drum process. Kintaro and Klim are whole milk products without any alteration as to the composition of milk. Morinaga is a dried product from a partly skimmed milk to which sucrose has been added. It is apparently neutralized before drying to promote the solubility of the product. Lactogen is apparently a whole milk product, but its lactose content is said to be modified.

The digestion experiment was made by adding 1 gram of pepsin to 50 cc. of reconstituted milk and incubated at 38°C. for 12 hours. After the incubation, the mixture was heated to destroy pepsin, and filtered. The nitrogen in the filtrate was determined. Knowing the amount of nitrogen of the original powder, the percentage digestibility was calculated. In parallel with dried milk a raw fresh milk was treated in the same way and its digestibility was also determined for comparison. The results are summarized in table 1.

TABLE 1

Apparent digestibilities of dried and raw milks with 2 per cent pepsin

RAW FRESH MILK		SPRAY PROCESS		DRUM PROCESS	
		Kintaro	Klim	Morinaga	Lactogen
	82.86	91.17	93.45	77.57	83.64
	82.86	89.98	93.46	86.56	82.43
	85.24	88.47	89.37	79.50	88.28
	86.81	89.03	89.37	79.50	88.28
	88.12	90.41	89.03	74.11	86.24
	89.49	88.73	90.12	72.26	86.83
	83.66	94.13	91.36	78.77	88.88
	82.61	94.13	91.36	77.12	87.69
	81.53	90.58	92.06	77.21	89.27
	81.01	92.18	91.01	77.24	88.69
		89.35	89.29	72.83	87.58
		88.80	90.45	72.83	87.58
		95.14	95.98	83.88	88.15
		96.26	95.98	84.51	88.75
		87.68	96.81	88.31	95.03
		96.01	96.26	88.31	95.03
		92.57	94.46	84.76	87.32
		93.15	83.86	85.42	87.32
		94.86	95.66	93.51	92.66
		95.42	96.22	92.88	83.83
Average	84.42	92.40	92.78	80.91	88.71

The average apparent digestibility of ten samples of the raw fresh milk with 2 per cent pepsin was 84.42 per cent, which was rather surprising, as it was less than those of the most of the dried milk. Klim showed the highest apparent digestibility, and Kintaro was the close second, both being over 92 per cent. Lactogen was the third, being 88.71 per cent, and Morinaga was the lowest of all, being 80.91 per cent. From these results it may be said that the drying of milk does not impair the digestibility of protein of milk with pepsin. Indeed the drying has rather the tendency of increasing the digestibility in certain cases. It is also noticeable that the spray processed products are much more digestible than the drum processed powders. The average digestibility of the spray processed powders was 92.59 per cent, while that of the drum processed products was only 84.81

per cent. This difference is not entirely due to the processes only, as it is seen that within drum processes Lactogen is much more digestible than Morinaga, the difference of the two being greater than the difference between the spray processed products and Lactogen. As it is well known that in peptic digestion, a certain amount of acid is necessary to be added, the acidity of the reconstituted milk must therefore be necessarily considered. As stated already Morinaga dry milk was apparently made by partly neutralizing the milk before drying. This must play an important part in interpreting this big difference in peptic digestibility. The acidity of reconstituted milk of different brands were as follows: Kintaro 0.171 per cent, Klim 0.176 per cent, Morinaga 0.045 per cent and Lactogen 0.135 per cent. It is noteworthy that the order of the peptic digestibility is exactly corresponding the order of the acidity, although they are not proportional. As the acid is always present in natural digestive juice, this preliminary experiment does not solve the problem, but gives only the degree of peptic action on milk protein. To imitate the natural digestion, the next experiment was carried out.

EXPERIMENT ON PROTEIN DIGESTION WITH PEPSIN AND HYDROCHLORIC ACID

The same brands of dried milk as in the preliminary experiment were used in this experiment. The powders were reconstituted in such a proportion that the resulting mixtures were similar to the normal milk in composition. Pepsin was added equally to all samples in an amount to constitute 1 per cent of reconstituted milk instead of 2 per cent as in the preliminary experiment. Different amounts of hydrochloric acid, *i.e.*, 0.1 per cent, 0.2 per cent and 0.3 per cent, were added to each brand to compare the efficiency of the peptic action. In this experiment the digestion was made in similar manner as in the preliminary experiment allowing five hours for peptic action. To the filtrate thus obtained in a flask, 10 cc. of 50 per cent trichloracetic acid solution were added and made to 200 cc. with water to precipitate any higher proteins such as casein, albumin, etc., still remaining in solution. This was filtered again and the nitrogen in the filtrate was determined, which may be termed as digested nitrogen. To see the extent of peptic cleavage of milk protein a further experiment was conducted with the last filtrates. To 50 cc. of the filtrate from trichloracetic acid 10 cc. of 2/3N H_2SO_4 and 10 cc. of 10 per cent Na-tungstate solution were added in a measuring flask and made to 100 cc. with distilled water. Upon mixing vigorously all albumoses, peptones and the like were precipitated. This was filtered and the filtrate contained amino and other soluble nitrogen compounds. The nitrogen in the final filtrate was determined. The difference between the original nitrogen and the nitrogen in the filtrate from trichloracetic acid was indicated as the indigested nitrogen. The nitrogen in the filtrate from trichloracetic acid was designated as the digested nitrogen. The difference between the latter and the nitrogen in the final filtrate was shown as the amount of albumose and peptone nitrogen.

In parallel with the above experiment the samples were treated with pepsin alone in order to see the importance of acid in protein digestion. All the experiments were carried out with tinned powders freshly opened. It was, however, anticipated that the digestibility may be changed after opening the tins. To see this relation the remaining powders in tins after using for the experiment were kept open for 4 weeks and the peptic digestion was again made in the same manner as in the freshly opened samples. The results are summarized in the following tables.

TABLE 2

Total nitrogen in 1 gram of dried milks

	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
	gm.	*gm.*	*gm.*	*gm.*
1	40.1357	42.0074	36.0941	38.9105
2	39.9098	41.5313	35.1998	39.1124
3	40.0488	42.5070	35.7102	39.2493
Average	40.0314	41.9152	35.6680	39.0907

TABLE 3

Nitrogen distribution after peptic digestion of dried milks, freshly opened

NITROGEN	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
	gm.	*gm.*	*gm.*	*gm.*
Indigested and in precipitates from trichloracetic acid	11.2014	12.3130	19.0972	20.1575
In precipitates from Na-tungstate	25.1288	26.2431	14.7474	16.7105
In final solution	3.7012	3.3591	1.8234	2.2227
Digestibility, per cent	72.02	70.62	46.46	48.43

TABLE 4

Nitrogen distribution after peptic digestion of dried milks from tins opened for 4 weeks

NITROGEN	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
	gm.	*gm.*	*gm.*	*gm.*
Indigested and in precipitates from trichloracetic acid	12.0915	13.3609	20.1672	21.3132
In precipitates from Na-tungstate	26.8127	27.1600	14.7768	16.7050
In final filtrate	1.1272	1.3943	0.7240	1.0725
Digestibility, per cent	69.79	68.12	43.46	45.48

TABLE 5

Nitrogen distribution after digestion with pepsin and 0.1 per cent hydrochloric acid

NITROGEN	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
	gm.	*gm.*	*gm.*	*gm.*
Indigested and in precipitates from trichloracetic acid	6.4050	7.1518	8.2170	8.5971
In precipitates from Na-tungstate	23.1932	24.9798	18.5110	21.1976
In final filtrate	10.4332	9.7836	8.9400	9.2960
Digestibility, per cent	84.00	82.94	76.93	78.01

TABLE 6

Nitrogen distribution after digestion with pepsin and 0.2 per cent hydrochloric acid

NITROGEN	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
	gm.	*gm.*	*gm.*	*gm.*
Indigested and in precipitates from trichloracetic acid	4.2632	4.4199	6.5572	5.6806
In precipitates from Na-tungstate	22.2710	23.6085	18.9552	22.0657
In final filtrate	13.4972	13.8868	10.1556	11.3444
Digestibility, per cent	89.85	89.46	81.62	85.47

TABLE 7

Nitrogen distribution after digestion with pepsin and 0.3 per cent hydrochloric acid

NITROGEN	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
	gm.	*gm.*	*gm.*	*gm.*
Indigested and in precipitates from trichloracetic acid	3.0464	3.6718	5.3502	4.3000
In precipitates from Na-tungstate	19.2746	22.7994	19.3757	22.6711
In final filtrate	17.7104	15.4440	11.9420	12.1196
Digestibility, per cent	92.39	91.24	87.80	89.00

Comparing tables 5 to 7 with table 3 it can distinctly be seen that the addition of hydrochloric acid greatly promotes the peptic digestion. Especially it is helpful in splitting the primary decomposition products of protein by pepsin such as proteoses and peptones into the final decomposition products such as amino acids and ammonia. It is also noticeable that in spite of a great improvement in digestibility of all samples of dried milks by addition of acid, there still remain distinct differences in digestibilities between different brands. The brand that showed a high digestibility with pepsin alone was also more digestible when acid was added.

In all cases Kintaro brand showed the highest digestibility, Klim coming closely next, Lactogen the third and Morinaga showing the least digestibility. The former two are spray dried products and the latter two belong to drum dried. Apparently it shows that the spray dried products are more easily digestible than the drum dried products. In consideration of the acidity of the dried milks, it is very likely that the variation in digestibility is not necessarily due solely to the processes of manufacture but may be due more to the treatment of milk before drying. Any neutralization of milk seems to be detrimental to the digestion of protein of dried milk.

Comparing tables 3 and 4 it can clearly be seen that the digestibility of dried milk is reduced after opening tins. It is suggestive, therefore, that the tinned dried milk should be used as soon as the tins are opened, not saying anything on the detrimental effect of changing in flavors and solubility. The percentage decrease in peptic digestion is calculated and is given in table 8.

TABLE 8

Percentage decrease of peptic digestion of protein of dried milks on standing after opening

DIGESTIBILITY	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
When tins opened	72.02	70.62	46.46	48.43
4 weeks after opening	69.79	68.12	43.46	45.48
Difference	2.23	2.50	3.00	2.95
Percentage decrease	3.10	3.54	6.46	6.09

The percentage decrease in digestibility after standing open is greatest for drum processed powders being nearly twice as great as that of the spray processed products.

EXPERIMENT ON DIGESTION OF PROTEIN WITH WHITE RATS

While there was a distinct difference in digestibility of protein of dried milk among different makes, the difference was even greater within the same milk with and without hydrochloric acid in peptic digestion. When hydrochloric acid was added the difference between different brands became much less, and this difference became still less with increased amount of hydrochloric acid. This necessitated an experiment to see if there is still any difference in digestibility of protein among different makes of dried milk with animals.

For experimental animals white rats were used. Baby albino rats after weaning were fed with clover, carrots, oats, casein and starch to the age of 61 days. Five of the same age and nearly of the same growth were selected and fed exclusively with dried milk for 56 days until the age of

118 days to let the animals adopt themselves to this food. The powdered milk was kneaded with a little water and given to the rats in form of a dough. From the 57th day of restricted feeding, or at the age of 119 days, the experiment was started. The animals were fed from weighed amount of dried milk. The experiment was continued for 4 days and faeces were collected for weighing and analyzing. At the end of this period any remaining milk was taken, dried and weighed. The remaining milk was subtracted from the amount given during the experimental period to obtain the amount consumed. The faeces collected were dried at 60 to 70°C. and the amount weighed. The dried faeces were ground in a mortar and sieved to take out any hairs or cotton fibers which may have adhered to the faeces. These remnants on the sieve were washed, dried, weighed and subtracted from the weight of dried faeces. By Kjeldahl method the nitrogen in the faeces was determined. Total nitrogen in the milk consumed minus the total nitrogen in faeces was taken as the digested nitrogen, or more correctly speaking the assimilated nitrogen. This, of course, does not give the exact figure for truly digested nitrogen, as in the faeces beside the indigested nitrogen there may be nitrogen of excreta of intestinal mucous membrane such as myosin and nitrogenous substances of pancreatic juice such as cholestin, lecithin, etc. Therefore the calculated digestibility is lower than the real. However, this error can not be very great, besides it is common to all. Therefore the results are comparable.

To eliminate any errors arising from individuality the animals were fed alternatively, except that the same dried milk was given throughout the experimental period for comparison. The digestion experiment was made in two periods in succession with the same animals on the same samples of dried milk. One feeding period was 4 days. In completion of the experiment with one dried milk, it was interchanged with the others in turn, giving 8 days for animals to adapt themselves to the new milk. This was repeated until all animals were given the equal chance for all dried milks under the test. Therefore, each of the 4 animals was used for 8 periods, and each milk was tested for two periods with all of the 4 animals. The results of the experiment are given in table 9.

The data in table 9 show that the digestibilities of different dried milks with albino rats do not differ as much as they did in artificial digestion. Still it can be seen that the spray dried products are more digestible than the drum dried products, and the order of the digestibility corresponds with that of the artificial digestion experiment. Kintaro was most digestible, Klim was the close second, Lactogen was the third and Morinaga was the least digestible. The index numbers taking the most digestible milk as 100 are Kintaro 100, Klim 99.78, Lactogen 99.03 and Morinaga 97.01. From these results it may be said that the system of drying does not give much difference in actual digestibility of protein of dried milk,

TABLE 9

Digestibility of dried milks with white rats

EXPERIMENT	SPRAY PROCESS		DRUM PROCESS	
	Kintaro	Klim	Morinaga	Lactogen
1	89.85	90.96	87.53	87.99
2	89.77	89.22	85.80	88.14
3	90.68	89.97	86.94	86.70
4	90.32	88.91	86.09	90.32
5	89.75	88.77	85.50	87.76
6	88.25	90.68	86.96	88.92
7	88.79	86.75	87.37	90.76
8	89.16	89.73	89.16	89.44
Average	89.60	89.37	86.92	88.73
Index number	100.00	99.78	97.01	99.03

but the treatment of milk before drying would have more influence. The neutralization of milk before drying to give a better solubility to the dried product is detrimental to the digestibility of the product. The difference of index numbers between Kintaro and Klim, both being the spray dried milk, is only 0.26, and that between Klim and Lactogen, the latter being a drum dried milk, is 0.71, while that between Lactogen and Morinaga, both being drum dried products, is as much as 2.02. The digestibilities of Kintaro at different periods of experiment with different animals varied from 88.25 to 90.68 per cent, the difference being 2.43 per cent. Those of Klim varied from 86.75 to 90.96 per cent, the difference being 4.21 per cent. Those of Morinaga varied from 85.50 to 89.16 per cent, the difference being 3.66 per cent. Those of Lactogen varied from 86.70 to 90.76 per cent, the difference being 4.06 per cent.

The effect of individuality of the animals played on this experiment may be seen from table 10.

TABLE 10

The effect of individuality of experimental animals to the digestibility of protein of dried milks

ANIMALS	SPRAY PROCESS		DRUM PROCESS		AVERAGE
	Kintaro	Klim	Morinaga	Lactogen	
A	90.50	90.090	86.230	90.100	89.230
B	89.81	89.440	88.265	88.340	88.964
C	89.00	89.725	86.515	88.565	88.451
D	89.04	88.240	86.665	88.510	88.114

There was some difference in digestible power of individuals. Rat A was apparently most powerful. In all cases except Morinaga, rat A showed the highest digestibility. On the average rat D was the least powerful.

With all animals except rat C, Kintaro was most digestible. Rat C digested Klim best. All rats except D digested Klim better than Lactogen. No rat digested Morinaga better than any other milks. The poor digestibility of Morinaga is therefore irrespective of the individuality of animals.

SUMMARY

1. The preliminary experiment shows that there is considerable difference in peptic digestion of protein in different makes of dried milk. With some makes of dried milk the protein is even more digestible than fresh milk. The order of the digestibilities from the highest is Klim, Kintaro, Lactogen, fresh milk, and Morinaga.

2. The protein digestibility of dried milk is influenced by the acidity of the reconstituted milk from dried milk. The digestibilities increase with acidities.

3. The addition of hydrochloric acid improves the peptic digestion in artificial digestion experiment. The digestibility is improved in all samples. It is, however, more striking with low acid milk.

4. Freshly opened dried milk is more digestible than that which was exposed for air after opening the tins. It is advisable therefore to open the tin of any dried milk when consumed.

5. Although the artificial digestion is greatly promoted by addition of hydrochloric acid and the difference between different brands is much reduced, the order of the digestibilities is not changed. The better digestible dried milk with pepsin alone is also better with addition of hydrochloric acid. In this experiment the order of the digestibilities from the highest is Kintaro, Klim, Lactogen, and Morinaga. The order of Klim and Kintaro was changed in this experiment from the result in the preliminary experiment in which the difference was very slight in favor of Klim. Otherwise the order is exactly the same.

6. The experiment with white rats shows much less difference in digestibilities among different makes of dried milk, but it also proves that the dried milk which shows a better protein digestibility with pepsin has the higher digestibility with white rats. The order of the digestibilities with white rats is exactly the same as the results in the artificial digestion experiment, Kintaro the first, Klim the second, Lactogen the third, and Morinaga the fourth.

7. The disadvantage in protein digestibility of Morinaga dried milk seems to be in its manipulation of milk in drying. Apparently the neutralization and addition of sucrose before drying impair the digestibility.

AN ECONOMICAL METHOD OF PRODUCING ACIDOPHILUS MILK

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Lactobacillus acidophilus grows poorly on artificial media and slowly in milk, which makes its production difficult under commercial conditions. The trouble comes usually from the facultative spore formers which are not killed by the temperature of steam under atmospheric pressure and which grow fast enough at 37°–40° C. to predominate easily over acidophilus, and the problem involved in the manufacture of acidophilus milk is to get rid of fast growing sporulating organisms.

The safest way, of course, is to sterilize the milk under pressure, and this is done by certain manufacturers. However, this involves great expense and the product made in this way is dark in color and possesses undesirable taste. Another method which has been recommended calls for the use of a good grade whole milk or skimmilk which is raised to near the boiling point and held at that temperature for one hour, cooled down to 37° C. and incubated at that temperature for four hours, which allows the germination of spores. The milk is then heated again to near the boiling point and held at the temperature another hour, cooled down to 37° C., inoculated with 2 per cent of its volume of a pure culture of *Lactobacillus acidophilus* and incubated at that temperature until it is coagulated. It is then cooled down, drawn into bottles and kept at a moderately cool temperature until it is consumed. This process is a lengthy one, requiring 9 to 10 hours before the milk is ready for inoculation, and this is mainly responsible for the excessive price of the product and limits its use by the general public.

The method reported in this paper has, over the one outlined above, two fundamental advantages, the one is a greater dependability and the other is a reduction of about 50 per cent in the time required for the completion of the process. It consists briefly in using clean, pasteurized skimmilk which is incubated for 2½ to 3 hours at 37° to 40° C. *without previous heating,* then heated to as close to the boiling point as possible, and held at that temperature for 30 minutes. The skimmilk is then cooled down as soon as possible to 37° to 40° C. and immediately inoculated with about 1 per cent of its volume of a pure culture of *Lactobacillus acidophilus* in the actively growing condition, and held at that temperature till it coagulates or, preferably, 3 or more hours after coagulation to insure a better keeping quality.

The method is very reliable when carried out properly and the significance of the various steps understood, and the author was able to con-

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sistently prepare perfectly good acidophilus milk, even after addition of one gram of soil per liter to the skimmilk. The principles involved are first to give the skimmilk an initial incubation at the same temperature at which it will be held after inoculation with *Lactobacillus acidophilus*. During that initial incubation, most of the viable spores present in the skimmilk, especially those which are most active at 37° C. and are most likely to give trouble, germinate and the vegetative cells destroyed by the subsequent 30 minutes heating near the boiling point. In this connection it is well to point out the importance of using skimmilk which does not already contain a large number of bacteria in the actively growing condition and which may grow enough during the initial incubation period to give the product undesirable flavors. Secondly, the inoculum must consist of a large number of actively growing cells. Milk cultures which have just coagulated, or preferably 2 to 3 hours before they coagulate, have been found to possess the required qualities. An important point in the process is a quick cooling after the skimmilk has been heated to near the boiling point and an immediate inoculation.

The method has been tested several months under commercial conditions and gave perfect results when the process was carried out properly. The product obtained in this way is lighter in color than that obtained by the now used methods, and possesses a slight cooked flavor.

SUMMARY

A new method of making acidophilus milk is described. This consists in incubating the skimmilk at 37° to 40° C. for 2½ to 3 hours, heating to as near the boiling point as possible for 30 minutes, quick cooling at 37° to 40° C., immediate inoculation with an actively growing culture of *Lactobacillus acidophilus* and holding at that temperature till coagulation takes place or a few hours thereafter. The method is more efficient and gives a better product than the one now in use and has the merit of cutting into half the time required to get the skimmilk ready for inoculation.

THE CORROSION OF METALS BY MILK

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INTRODUCTION

Milk is the most important food for human consumption. According to Holm (1) there was produced in the United States in 1926 53,000,000 tons of milk with a market value of about $4,000,000,000. By way of comparison, the value of the automobile industry is placed at about $3,500,000,000 and steel at $3,000,000,000.

Cooperative milk depots, pasteurizing plants and commerce have ordained the use of large installations of metal equipment. A very large proportion of the milk produced in America today is pasteurized in metal equipment.

In strong contrast to its very mild action on the organs of the human body, the effect of milk on most metals is quite pronounced, in fact, the corrosion of metals by milk is one of the most serious corrosion problems facing modern civilization and industry.

IMPORTANCE OF THE MILK CORROSION PROBLEM

Not only does the corrosion of metals by milk become an economic problem from the point of view of equipment replacement, but it is important also from the marketability and physiological factors involved. Improper equipment may lead to contamination of the milk by the products of corrosion to such an extent that not only is the flavor affected, but also the food value. The flavor may be affected by simple metal contamination, giving the characteristic metallic taste, or by the indirect action of the dissolved metals on the microorganisms in the milk, resulting in the "mealy" flavor (2).

A considerable amount of information has been obtained on the effect of metals in foods upon animals and human beings. The present paper is not intended to be a physiological treatment of the subject. However, this much can be said—nature has given us in milk a food which has been unchanged over thousands of years; it is hardly to be expected, in spite of many recent investigations indicating the contrary for iron and copper, that the addition of foreign metals in solution in the milk, as products of corrosion of equipment, will improve this food. Does it not seem logical that a milk which has been pasteurized in the proper metal equipment and has retained its original metal and other constituents without the intro-

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duction of foreign materials will be superior to a milk which has been relieved of part of its metallic contents or has been contaminated by foreign metals?

PREVIOUS INVESTIGATIONS

The corrosive action of milk on metals is by no means a new observation. It has long been known that milk in contact with iron and copper will not only acquire a metallic taste, but corrode these metals readily.

Supplee and Bellis (3) found that just a few bronze joints and tees in a pasteurizing unit would increase the copper content of the milk. The action of milk upon copper and other metals has been studied by Donauer (4), Rice and Miscall (5), Quam, Soloman and Hellwig (6), Miscall, Cavanaugh and Carodemus (7).

Nickel and Monel metal have been studied by McKay, Fraser and Searle (8), Flowers (9), and others. Chiara (10) studied these metals along with their zinc alloys.

Hunziker, Cordes and Nissen (11) have made a very extensive study of the action of milk upon a series of metals and alloys. These investigators and others have tested copper, nickel, aluminum, iron, tin, zinc, Monel (nickel-copper-iron alloy), German or nickel silver alloys, "stainless" alloys, etc.

METHODS OF INVESTIGATION

Almost all of the investigators determined the corrosion of the metals in milk by subjecting weighed samples of the metals to the action of milk for definite periods of time at certain temperatures. After the exposure the metal sample would be weighed again and the amount of corrosion estimated from the loss in weight. As will be shown later and has previously been pointed out by students of corrosion, loss in weight is not always a good criterion of the degree of corrosion. The accuracy of a loss-in-weight determination can, however, be greatly improved upon by visual or microscopic observations.

Almost all of the earlier investigators were unanimous in attributing the corrosion of metals by milk to acid corrosion, that is, substitution of hydrogen for metal.

STATUS OF METALS IN MILK EQUIPMENT

Hunziker, Cordes and Nissen (11), as a result of their recent detailed investigation, align the metals they tested into the following four groups, according to relative merit:

1. Allegheny Metal, tin, and heavily tinned copper.
   These metals have no effect on flavor and show maximum resistance to corrosion and tarnishing. The tin coating on copper must be heavy and intact.

2. Nickel, aluminum, and manganese aluminum alloy.
   These metals are not entirely satisfactory in high acid milk products. Furthermore, the nickel tarnishes readily and the aluminum is sensitive to alkali washing powders.
3. Monel Metal, Enduro, Ascoloy, and nickel silver.
   Monel Metal tarnishes appreciably in milk products. In these tests it had only slight effect on flavor. Enduro and Ascoloy are sensitive to high-acid products and their performance in dairy equipment is uncertain. Nickel silver tarnishes severely and was injurious to flavor in the majority of milk products.
4. Tinned iron, copper, galvanized iron, iron, and zinc.
   With the exception of properly tinned iron, this group is unfit for use in contact with milk products. Tinned iron is equally so whenever iron is exposed.

The above tabulation is not strictly in accordance with our own findings, notably in the case of the 18–8 chrome-nickel iron alloys. We group the metals and alloys as follows (see table, page 18):

(1) 18–8 alloys: (Allegheny metal, Enduro KA_2, etc.) Hyblum, tin, heavily tinned copper and chromium plated copper or nickel.
(2) 95 Copper–5 Nickel alloy, 12–13% chrome-irons.
(3) Monel, nickel-silvers, nickel, copper, aluminum, manganese, silicon-copper alloy, Ambrac.
(4) Iron, tinned iron, galvanized iron, zinc.

The basis for our tabulation will be evident from the experiments recorded in the following pages.

THE NATURE OF CORROSION

Before discussing the nature of corrosion by milk, it seems logical that one should first understand the fundamentals of corrosion by simple solutions. The electrochemical theory of corrosion is today almost universally accepted. When a metal goes into solution one can say then that it is the result of an electrochemical action. As the metal goes into solution and passes from a zero charge, as the metallic state is designated, to positively charged ions, an equivalent amount of electricity must pass from the solution to the metal so as to neutralize the charge. In the case of acid corrosion this may be expressed as follows:

$$M^o + H^+Cl^- \longrightarrow H^o + M^+Cl^- \qquad (1)$$

In the case of corrosion by metallic salts this may be expressed as follows:

$$M^o + Cu^{++}SO_4^- \longrightarrow Cu^o + M^{++}SO_4 \qquad (2a)$$

$$2\,M^o + Cu^{++}SO_4^{=} \longrightarrow Cu^o + M_2^+SO_4^{=} \qquad (2b)$$

In the case of corrosion due to the presence of oxygen, this may be expressed for copper as follows:

$$2\ \text{Cu (metal)} + 2\ \text{O} = 2\ \text{CuO} \qquad (3a)$$

then

$$2\ \text{CuO} + 2\ \text{HCl} \rightarrow \text{CuCl}_2 + \text{H}_2\text{O} \qquad (3b)$$

Before reactions 1 and 2 can proceed, the concentrations of acid and metallic salts must be high enough by Nernst's equation to make that reaction possible, thus at low concentrations of acid, or metallic salt, corrosion by these means may not be possible. Furthermore, in order that case (1) may be satisfied hydrogen must either evolve or be removed with oxygen, otherwise corrosion cannot proceed. This is the well-known phenomenon of over-voltage polarization and oxygen acts as a depolarizer.

The cases just presented are subjected to a number of factors which affect the speeds at which they proceed, such as chemical and physical conditions of the metal, kind of solvent, circulation, etc.

A point of note is that oxygen may sometimes, contrary to general belief, inhibit rather than assist corrosion. Thus, if the rate of oxygen supply permits the formation of slightly soluble oxide films, the metal is said to become passive and resists solution. This is frequently the case with iron and nickel, as investigated by Evans (12).

Every corrosion problem is a problem in itself, many times appearing to overthrow the fundamental concepts gained from other experiences. The problem of corrosion of metals by milk is one like this. Why milk should have such a profound effect on metals has never before been satisfactorily explained.

THE NATURE OF CORROSION BY MILK

Milk is known to be a complex body containing both organic and inorganic radicals. Ordinary milk tends to be slightly acidic, having a pH at room temperature of 6.6–6.7 when sweet, to 4.6 when sour. It is evident, then, that unless extreme precautions are taken immediately after the milk is drawn from the cow, it will become slightly acidic due to the lactic acid produced by bacteria (13). Increasing the temperature of milk increases the acidity (14), consequently this factor may be one of the explanations why the corrosion rate of metals in milk increases at the higher temperatures. Quam (15), as well as others, have found that the corroding action of milk on metals passed through a maximum at about 80°C., as the temperature of the milk was gradually increased.

It is quite evident that the acidity of milk is too low for Equation (1) to proceed in the absence of oxygen. That this is true can be ascertained from the results of Emery and Henley (16) who found that milk had very little effect on some metals in the absence of oxygen. As the temperature of milk is being increased, the oxygen content decreases, and the acidity, as mentioned before, increases and at the same time viscosity decreases (17). The first factor being unfavorable for corrosion above 80°C. must overshadow the favorable effects of viscosity and pH, causing a drop in the solubility rate curve. It is well known that above 80°C. the oxygen content of water, which may be compared to milk, decreases to almost zero.

The presence of a large amount of dissolved oxygen at the lower temperature might even act to inhibit the corrosion of such metals as nickel which readily forms protective oxide films. This may be one explanation why the average nickel pasteurizing unit does not corrode very readily in the cold sections of the equipment, yet frequently does corrode in the regenerator where a large amount of the oxygen is driven out of solution and leaves an insufficient amount to promote the formation of passive films.

Donauer (4) has calculated that the corrosion rate for most metals is ten times greater at pasteurizing temperature than at room temperature. That a certain amount of oxygen is necessary may be true for the type of corrosion as indicated by Equation (1), and, again, it is quite possible that an excess of oxygen will inhibit the corrosion of metals like nickel.

THE FINK THEORY OF CORROSION BY MILK

Fink attributed the corrosion of nickel by milk to the copper in the milk (18). It is the copper present in all normal milk that is responsible for a great deal of the observed corrosion. We may express the theory of base metals according to the Equation (2):

$$\mathrm{Ni} + \mathrm{Cu}^{\cdot\cdot} \longrightarrow \mathrm{Ni}^{\cdot\cdot} + \mathrm{Cu}$$

It is a well-known fact that all normal milk contains copper to the extent of 4–6 parts per ten million (19). This copper content is easily raised, as previously mentioned, by the presence of bronze, brass or copper fittings and equipment in contact with the milk. Moreover, the influence of oxygen is here very slight.

This type of corrosion, due to metal substitution, is similar to acid corrosion except that it is influenced by a greater potential, that is, in equimolecular concentrations of copper and hydrogen ions, nickel has a greater tendency, by about 0.3 volts, to go into solution in the presence of copper (20). This potential difference is, of course, influenced by the respective activity concentrations of hydrogen ions and copper, and may be mathematically expressed at 25°C. as:

$$E_1 = \left(-.23 + \frac{0.059}{2} \log C_{Ni}\right) - \left(+.34 + \frac{0.059}{2} \log C_{Cu}\right)$$

$$E_2 = \quad \text{``} \qquad\qquad \text{``} \qquad - (\ 0 + 0.059 \log C_H)$$

Subtracting,

$$E_3 = -.34 - \frac{0.059}{2} (\log C_{Cu} - 2 \log C_H)$$

These equations demonstrate theoretically that the corrosion of nickel pasteurizing equipment is influenced more by the copper concentration of the milk than by the hydrogen-ion concentration. Moreover, hydrogen has a greater over-voltage than copper, making for an even greater difference. The argument may be advanced that the copper in milk is bound up into complex combinations and does not present the "activity" that the hydrogen ions do. That this argument is untenable will be shown later.

Rice and Miscall (5) have shown that, as milk was being handled in nickel cream separators, the copper content decreased. This decrease in copper could not have taken place without solution of nickel. A close examination of milk-shake mixers made of nickel will give evidence of a dark coloration that does not wash off. By taking a badly corroded nickel regenerator tube and dissolving this black coloration in nitric acid, we were able to electroplate copper out of the resulting solution.

LARGE SCALE TEST: CORROSION DUE TO COPPER

With this knowledge, and not disregarding the effects of acid corrosion and of oxygen, a test was made upon one of the pasteurizing plants of New York City. This plant was similar to the many other plants of the city; milk was stored in enamelled tanks, the pasteurizing equipment was constructed of nickel and copper with the usual bronze joints.

In this plant test samples of milk were taken from thirteen different locations or sections of the pasteurizing equipment. These samples were taken by a disinterested party and labelled by numbers, consequently, when they arrived in our hands, our only guide were the numbers. We had no information on any sample as to whether it had been in contact with either cold or hot metal or what this metal was.

DETERMINATION OF COPPER IN THE MILK SAMPLES

The samples of milk were then analyzed by the xanthate, colorimetric method. Many trial analyses on known water blanks containing organic matter were tried before the errors of the method were under control and the technique mastered. Duplicate samples of milk of 100 cc. each were slowly evaporated and ashed in 200 cc. silica evaporating dishes. The contents of the evaporating dish were protected from possible gas flame contamination by means of an asbestos collar.

Ashing in an electric furnace was tried but erratic results were obtained due to copper volatilization at the higher temperatures. Electric furnace ashing should no doubt be satisfactory if the temperature could be controlled within reasonable limits.

It was soon noted that the silica became covered with a dark crust which resisted later solution. This crust was found to dissolve in hydrofluoric acid. Consequently, after each ashing a few drops of hydrofluoric acid were added to the hydrochloric acid when the ash was to be dissolved. From here on the usual method was applied, namely, evaporation of the dissolving hydrochloric acid solution in water, addition of ammonia, filtration, and neutralization to the same pH. The identical quantities of water, acid, and ammonia were used with each milk sample and the analytical procedure, time, temperature, etc., were as nearly identical as possible. These preliminary steps were then followed by the colorimetric xanthate comparison with known standards. All comparisons were made in the same light and under the same conditions of temperature.

The above procedure in the copper determinations, although exceedingly laborious and requiring considerable care, gave very good checks and enabled us to predict whether the milk had gone over nickel or copper, or copper alloy. When it had passed over nickel, the copper content would decrease by reaction (2a), and when it had come into contact with copper, the copper content would increase by reaction (3). After completing the analyses a description of each sample of milk was furnished us, including temperature, metal contact, etc. In each one of the thirteen cases the amount of copper found agreed almost precisely with what was to be expected. We could thus tell in advance from the copper analyses whether the milk had just passed over copper or nickel metal. In one case only, where chromium plated copper equipment had come into contact with the milk, a very slight change was noticed which in due fairness could be attributed to a slight experimental error. A striking illustration of a series of tests was in the case of one of the regenerators. This regenerator was constructed as follows: The first six tubes were of pure nickel, followed by 8 copper tubes and then again by 16 nickel tubes. The analysis of the milk at the inlet of this regenerator indicated a copper content of 0.43 p.p.m. After passing through the six nickel tubes, the copper content dropped to 0.31 p.p.m. At the outlet of the eight copper tubes, the copper content was found to have increased to 0.76 p.p.m. At the outlet of the remaining tubes of nickel, the copper content of the milk was found to have dropped to 0.43 p.p.m.

These results are only examples of how the copper content varied in the entire pasteurizing equipment. A number of the nickel tubes exposed to the milk showed deeply corroded areas, indicating loss of nickel metal, and, although no analysis was made of the nickel content of the milk, it is safe to assume that the nickel content was accumulative because of the absence of any less noble metal for the nickel to plate out upon.

The copper analyses do, no doubt, vary from day to day, depending upon the rate of pasteurizing and temperatures, but will, we believe, always be in the same sensible order as found in this particular case.

DIFFERENCES IN CORROSION RATES

A careful corrosion study of a pasteurizing plant of this type revealed to us that the corrosion of the nickel is most severe in the regenerators. In almost all cases the most severe corrosion was found to have occurred in this portion of the equipment. Here the corrosion is so pronounced as to deeply pit the tubes. The nickel equipment in the other sections of the plant is not corroded to the same extent as the regenerator tubes.

A THEORY OF THE DEGREE OF CORROSION

As previously mentioned, in the presence of oxygen nickel has a tendency to form a protective film of oxide which resists corrosive action. Brown, Roetheli and Forrest (21) have recently made some very interesting measurements on the initial corrosion rates of several metals among which were nickel and copper. The results they obtained showed that in the presence of aerated water nickel went into solution much more slowly than copper. In other words, the oxide film that must first form in the case of nickel is less soluble and protects the underlying metal better than the copper oxide film. It must be remembered that in the presence of oxygen the potential of the metal is in reality the potential of the metal oxide. Too liberal a use of the electromotive series is then not permissible.

As these effects of oxygen and oxide films in water are no doubt approximately the same in the case of metals in milk, many apparent discrepancies can be explained. At first the milk is cold and contains a liberal amount of oxygen; then, as it passes through nickel equipment, very little action takes place—in fact, if a few copper tees and pipes are present, the copper content will quite likely increase instead of decrease. This was found by Supplee and Bellis (19) and confirmed by our own experiments. As this milk is fresh, the pH is high, generally around 6.6–6.7; consequently, acid corrosion is insignificant.

After the milk is heated to the pasteurizing temperatures and transported through pipes to the regenerators, a change in the milk takes place. As previously mentioned, the viscosity decreases and the pH decreases with a higher temperature. Above all, the amount of oxygen dissolved in the milk is decreased considerably: Consequently, nickel does not readily form a protective film in the presence of the low oxygen concentration. When the oxygen content of the milk drops below a certain value, the corrosion of the nickel increases, the rate for the formation of a continuous, protective film now being far below the corrosion or pitting rate. At the same time as the oxygen or air in the milk is eliminated, any copper that is present in the milk has a tendency to pass from the cupric (Cu^{++}) to the cuprous (Cu^{+}) state (22). Increasing the temperature also favors this tendency. When copper is in the cuprous state it has a greater tendency to plate out of solution because $Cu^{+} \rightarrow Cu^{0} - 0.51$ volts; therefore, in a heated solution of copper, or in heated copper containing milk, in the presence of a less noble metal like nickel, a greater corrosion rate, by metal substitution, will result than in the cold solution.

THE MECHANISM OF THE DISPLACEMENT REACTION

The action in the case of milk upon a metal like nickel may be visualized as follows: To begin with, a small amount of nickel goes into solution and at the same time copper metal plates out of solution. A couple is then formed on the surface of the nickel and more nickel goes into solution, resulting in a small pit on the surface of the tube. This may be called the primary stage of the corrosion.

In the second stage of corrosion another factor may enter in. Bacteria called thermophiles (23) are present in most milk, and, although physiologically harmless, thrive at high milk temperatures, and decompose the lactose to lactic acid. These thermophilic bacteria, or spore formers, are aerobic (oxygen consumers) and "delight in living in the milk solids that might cook to the sides of the pasteurizing equipment" (24). These bacteria have a tendency to seek the crevices of the tubes and resist all but the most thorough efforts of cleansing.

It is quite evident, then, that these bacteria will settle in the pits formed by the first stages of corrosion. After they have accumulated and multiplied, each pit will be an active corrosion center. The oxygen content of the pit will decrease, the acid concentration increase, and nickel will go into solution. This might be called the second stage of the corrosion.

The actual plant observations show that when the regenerator tubes are made of nickel they are badly pitted.

It is of interest to note that the interior of these tubes is dark in color. To test for the presence of copper deposited on the nickel, the inside of a tube section was swished with nitric acid. The tube brightened and the resulting solution was electroanalyzed. Immediately a copper deposit formed on the platinum cathode, thus proving that the dark colored deposit on the nickel tube wall was partly, if not completely, due to copper.

MILK CORROSION TESTS WITH OTHER METALS

In order to find a metal or alloy which would resist the corrosion of milk at low and high temperatures and which would be more suitable for pasteurizing equipment than any metal now in use, a new series of experiments was initiated. Small samples of metals and alloys were subjected to raw milk for definite lengths of time and at controlled temperatures. The milk was analyzed for copper before and after the experiment. Besides this, no other test except a visual examination was given each metal specimen after an experiment.

On the basis of the findings recorded above, it is obvious that, as a metal is being substituted for copper, the weight loss or gain will give very little information or indication as to the corrosion performance. On the other hand, an analysis of the solution or milk will certainly tell the story of the corrosion.

Inasmuch as copper has a very great tendency to go into solution in the presence of oxygen, and nickel has a tendency to displace copper from solution, it was thought that some alloy of the two metals might be made in which the tendency of one metal would counteract that of the other. We were encouraged in this belief since we had observed that nickel-copper alloys high in copper would, at any well defined temperature of the milk, lose copper, and alloys of the two metals high in nickel lose nickel. Accordingly, there ought to be an alloy of the two metals which at, say, pasteurizing temperatures would satisfactorily resist the corrosion by milk. The value of such an alloy is evident: corrosion would be eliminated and the copper content of the milk would remain undisturbed. Since Monel metal (approximately 28% Copper and 69% Nickel) is known to be corroded by milk, resulting in a milk high in copper, alloys of 5, 10, 15 and 20 per cent copper, balance nickel, were made up. These alloys were carefully made, poured into clean molds, given the same uniform heat treatment, and carefully polished.

Of this polished area 50 sq. cm. was then exposed to 200 cc. of raw milk at 40°F. (4.4°C.) for 60 hours. The raw milk previous to exposure showed copper to the extent of 0.50 p.p.m. The results of the analysis are as follows:

COMP. OF Cu-Ni ALLOY		TIME OF EXPOSURE	TEMP. °F	Cu CONTENT OF MILK	
% Cu	% Ni	Hrs.		Initial	Final
				p. p. m.	*p. p. m.*
20	80	60	40	0.5	1.25
15	85	60	40	"	1.02
10	90	60	40	"	0.65
5	95	60	40	"	0.52
0	pure nickel	60	40	"	0.10

The metal specimens were all tarnished to about the same degree. It was quite evident, after the samples had been cleaned and then exposed to hot milk and had given the same trend in results, that no alloy of nickel and copper could be successfully employed at both temperatures to resist corrosive action of milk. The alloy of 5% Cu and 95% Ni did maintain a good copper balance in the milk at 40°, but not at higher temperatures.

All of the samples showed the characteristic discoloration due to the cementation of copper. It might seem peculiar that copper will plate out and/or go into solution from the same specimen. We may attribute this to a specific local action. When nickel and copper are melted together they form a simple, solid solution. Upon solidification, then, both nickel and copper will be in the space lattice. The copper in the milk will then substitute for the nickel in the alloy's surface. The result is a specimen presenting only a copper surface. As the concentration of oxygen builds up, the oxygen-liquid corrosion is now diverted to the copper, causing its solution, which results in nickel being uncovered and subjected to the attack. This cycle continues, and, as in the case of Monel metal, solution rate is faster than ordinarily.

OTHER ALLOYS INVESTIGATED

Allegheny metals, Nirusta, and other 18–8 alloys suggested themselves as possible substitutes for copper-nickel equipment. The results of the corrosion tests on these chrome alloys are given in table 2.

Alloys of nickel, copper and zinc have been recommended frequently for milk pasteurizing equipment. Recently the alloys Ambrac A and Ambrac B have been favored in some installations. These were tested by us and found to go into solution, bringing up the copper content, but leaving a smooth surface rather than a pitted surface. The results of these alloys, when corroded by milk, are also included in table 2.

The last alloy of promise that was studied in the research so far carried out was Hyblum. Hyblum is a high-aluminum nickel-aluminum alloy. It is very white, and light in weight. As the results in table 2 show, it gives promise of being good metal to use. However, it does not stand up well under the action of cleaning compounds that are commonly used in dairies.

TABLE 2

METAL	APPROXIMATE COMP. OR KIND	TIME OF EXPOSURE	TEMP. °F.	CU CONTENT OF MILK	
		Hrs.		Initial	Final
				p.p.m.	p.p.m.
Ambrac A	Cu 75, Zn 5, Ni 20%	24	145	0.55	2.05
Ambrac B	Cu 65, Zn 5, Ni 30%	24	145	0.55	1.15
Monel	30 Ni 70%	24	145	0.55	0.83
Nickel	Pure, rolled plate	24	145	0.55	>0.10
Hyblum	Al 95 Ni 5	60	40	0.50	0.50
Enduro KA$_2$	Ni 8, Cr 18, Fe 74	24	145	0.55	0.55
Allegheny	" " "	24	145	0.55	0.50

SUMMARY AND CONCLUSIONS

Investigators in the past have shown that:

(1) Copper, naturally present in small amounts in milk and other foods, is a physiologically necessary and important constituent (25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35).

(2) The natural copper content of milk varies between 0.3 and 0.7 parts per million.

Our own experiments demonstrate conclusively that:

(3) Copper, nickel and copper-nickel alloys, when exposed to milk during pasteurization, may bring about an increase above 0.7 p.p.m. or a decrease below 0.3 p.p.m. in the copper content of the milk.

(4) This change in the copper content of the milk is more pronounced at elevated temperatures.

(5) When milk in contact with nickel apparatus loses copper, this metal is precipitated out and nickel goes into solution.

(6) Nickel corrodes in milk more readily in the *absence* of oxygen.

(7) Copper corrodes in milk more readily in the *presence* of oxygen.

(8) High chromium nickel (18–8) iron alloys, "Hyblum," chromium-plated copper and chromium plated nickel are very resistant to corrosion by milk and are satisfactory for dairy equipment on this account.

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A STUDY OF THE GESTATION PERIOD OF HOLSTEIN-FRIESIAN COWS[1]

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Investigators have made occasional studies of the duration of gestation of cattle for over a century. Most of these studies have been made with a relatively small number of animals and because of the extreme variation found by some of the earlier investigators, it seems quite probable that some of the records to which they had access may not have been as accurate as could be desired. Those who have studied this subject more recently have, in general, found less variation and have arrived at conclusions that are more uniform.

The length of the gestation period has interested stockmen for many years, because a knowledge of the time to expect the birth of the calf enables proper preparation, such as drying off the cow and getting her in the proper physical condition for parturition and subsequent lactation.

The duration of gestation may be, to some extent, an index of the sexual health of the breeding herd. It is believed by many stockmen that any calf that is born following a gestation considerably shorter than the average is not liable to develop into an animal of superior merit.

Early investigators (1), (2), (3), (4) observed gestation periods varying from 210 to 353 days with averages ranging from 280 to 288 days. It is difficult to understand how periods varying as much as 50 per cent of the mean could be normal, although the upper and lower limits of normal gestations have not been definitely established. Many stockmen question the normality of gestation periods shorter than 270 days and it is quite possible that many reported periods exceeding 290 days may be the result of later services that were not recorded.

Williams (5) has given 285 days as the standard with a normal variation of five days above or below that figure. No authority is given for his assumption that 285 days is the ideal length of gestation in the cow.

Wing (6) reports on his observation of 194 gestation periods in the Cornell University herd. The shortest gestation period observed by Wing was 264 days and the longest 296 days with an average of 280 days regardless of the sex of the offspring. The gestation periods where twins were born averaged five days less than the general average and eight days less than the average of the single births of the same cows.

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Fleming (7) summarizes 1,062 observations made at the Agricultural School of Saulsaie, and by Blaine, Tessier, Grille, and Furstenberg. Fifteen were pregnant for less than 241 days, 52 from 241 to 270, and 32 beyond 301 days with an average of 283 days.

McCandlish (8) presents data collected from the dairy herd at the Iowa State College. His summary is based on 369 gestation periods with a mean of 280 days. The length of the gestation period was not affected by the sex of the calf, the breed of the cow, age at calving, or season of freshening.

Contrary to McCandlish's findings, Fitch, McGillard and Drumm (9) found a difference in the length of gestation periods between breeds. The average for Jerseys was 284.3 days; for Guernseys 283.0 days; for Ayrshires 284.6 days, and for Holsteins 281.0 days.

Copeland (10) in studying 1,075 cases of Jersey cows found an average length of gestation of 278.5 days. He observed that there was an equal chance of a cow calving any time between the 271st and 285th day. His data showed that the age of the dam had no effect on the length of gestation and that the male calves were carried approximately one day longer than the female calves.

INVESTIGATIONAL

This study was undertaken to determine, insofar as possible with the material available, the average length of the normal gestation period of Holstein-Friesian cattle, the variations that occur, and the causes for such variations. It was believed that data on a large number of gestations might give rather definite information on many points that had been indicated in previous investigations, but had not been substantiated for lack of data on a sufficient number of cases.

The data used were obtained from the herds of the Carnation Milk Farms at Carnation, Washington; the Western State Hospital at Fort Steilacoom, Washington; and the State College of Washington at Pullman, Washington.

Data were obtained on 2,910 gestation periods. Seventy-eight of the animals included were grade Holsteins and 2,832 were pure bred Holsteins. Twins were dropped following 86 of these gestation periods and are considered separately in the presentation of the data.

The term "gestation period" is usually understood to include the time from the effective service of the cow by the bull until the birth of the calf. Mumford (11) gives the following definition:

> "The period of development from the fertilization of the egg by the sperm cell until the birth of the fully developed offspring capable of independent existence outside the body of the mother is known as the period of gestation."

Ovulation and the fertilization of the ovum usually occurs from 30 to 60, or more, hours after the cessation of the mating period. It is not possible to use Mumford's definition of the gestation period in this work, because it is not known just when fertilization of the ovum takes place in each pregnancy.

The term "gestation period," as used in this paper, includes the period from the time of service to the birth of a living, fully developed calf including both the day that the cow was bred and the day that the calf was born. In collecting these data, no gestations that ended in the dropping of a dead calf were included. Whenever a calving was marked as abnormal on the breeding record, the gestation period was excluded. Where the necessary information was available, all gestation periods that were terminated by the birth of a living calf were included.

The average length of the 2,824 gestation periods studied that terminated with single births was 279.9 ± 0.063.

Table 1 shows the distribution of the cases included in this investigation.

Table 1. Distribution and Frequency of Gestation Periods

Age years	Sex	Days in Gestation Period																																		
		262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296
1	Female									1										1		1														
	Male												1		1	1	1	1		2	1	2		1	1											
2	Female	1				2	1	1	1	10	1	13	11	19	19	31	24	22	26	19	20	22	21	15	10	11	7	2	2	1	2		3			
	Male					2		1	3	4	6	8	16	8	14	17	21	25	26	16	32	22	18	19	14	13	8	4	6	5	2	2	1			
3	Female		1				1	2	1	3	5	7	12	11	12	20	11	21	19	19	12	29	13	6	14	12	4	3	1	1						
	Male		1			2	1	1	4	5	3	6	9	9	12	11	20	16	21	21	23	28	17	18	10	12	5	2	3	3			1	1		1
4	Female					2	1	1	2		7	5	7	9	8	16	14	16	21	28	13	10	13	7	8	5	5	2	4	2	2					1
	Male							2	1	1	1	4	4	6	10	7	15	16	10	14	17	21	18	13	9	5	7	2	7	2	2		2		1	
5	Female				1		1			3		2	6	8	5	9	14	11	14	11	8	7	8	11	4	6	5	1	2		1	1			1	
	Male						1		1	2	1	2	3	8	8	5	7	11	10	10	12	9	14	6	6	10	14	3	2	3	1	1	2		1	
6	Female									1	1		3	1	7	7	15	13	8	7	12	10	4	5	7	6	5	1	3	1	1		1			
	Male			1					1	1	1	1	2	2	4	6	10	8	11	8	6	7	15	9	11	4	4	2	2	3		1	1			
7	Female										3		2	2	6	10	5	3	6	10	7	7	10	4	4	5	6		2	1		1				
	Male							2		1	1	5	2	1	6	4	2	7	13	7	9	8	8	6	5	2	2	4	1	3	2		1		1	
8	Female								1	1	1	3	3	2	3	9	8	8	5	9	7	7	4	5	6	2	1									
	Male								1	2	1		1	4	3	6	3	3	6	7	7	9	11	7	4	1	1	4	1	4				2		
9	Female							1			3	2	3		2		3	3	5	4	6	2	3	4	6	3	2	3		1				1		
	Male								1	4		1	1	3	1	5	8	7	4	1	6	5	5	5	6	3	4			1		1				
10	Female											2	2	1	1	5	1	2	3	2	7	1	5	3	2	3	1									
	Male							1		1	1			5	1	4	3	3	2	1	3	3	6	5	3	4	2	2	2	1						
11	Female							1					2	2	2	1	4	4	3	1	5	1	3	3	1	2			1					1		
	Male									1	1	1	1	1	1	2	1	1		3	1	1	3	1	4	1	4									
12	Female										2		1		1	2	1		3		2	6	1	2		2	1									
	Male																2	1	4	1	2		1		2		1		1							
13	Female															2		2		1	2	1				1										
	Male															1	2			2	2		1				1		1	1						
14	Female																			1		1					1									
	Male															1	1								1			1								
15	Female																1		1																	
	Male																		2			1						1								
Total Females		1	1		1	4	4	6	5	19	23	34	52	55	67	114	101	105	114	113	101	106	85	65	62	58	38	12	15	7	6	2	4	1	1	1
Total Males			1	1		4	2	7	12	22	16	29	40	47	61	70	96	99	109	93	121	116	117	90	76	55	53	25	26	26	7	5	6	3	3	1
Total Both Sexes		1	2	1	1	8	6	13	17	41	39	63	92	102	128	184	197	204	223	206	222	222	202	155	138	113	91	37	41	33	13	7	12	4	4	2

The shortest period observed was 262 days. The total number of periods less than 270 days is only 49, or 1.7 per cent of the total number. The number occurring from 270 to 279 inclusive is 1,273, or 45.1 per cent. From 280 to 289 days inclusive, there are 1,427 cases, or 50.5 per cent. There are 75 cases, or 2.7 per cent, that are for 290 days, or longer, with 296 days as the longest gestation period observed.

The distribution on each side of the mean is quite uniform, as we find that there are 1,296 cases, or 45.9 per cent, that were carried for 281 days or longer and 1,322 cases, or 46.8 per cent, carried for 279 days or less.

Fewer calves were born on the 280th day, which is the mean, than there were on the 279th, 281st, or 282nd days.

One thousand seventy-seven calves, or 38.1 per cent, were dropped during the five days from 278 to 282 inclusive. The period of seven days, which includes three days each side of the mean, or from 277 to 283 days inclusive, includes 1,476, or 52.3 per cent, of the total cases. Two thousand eighty-one cases, or 73.7 per cent, are included in the period from 275 to 285 days inclusive, which includes five days above and below the mean.

The influence of the sex of the calf upon the duration of gestation is a question about which there has been a considerable difference of opinion. Many investigators have found that the average gestation period was the same regardless of the sex of the calf, while others have found that the male calves are on the average carried in dam longer than the females.

The average length of gestation periods in days in the 1,441 cases where males were dropped was 280.4 ± 0.090. One thousand three hundred eighty-three females were dropped following gestation periods that averaged 279.4 ± 0.087. This difference of exactly one day is certainly not large, but statistically significant, as it is eleven times the probable error. A thorough study of the data presented can hardly fail to reveal that there seems to be a well defined tendency for the male calves to be carried longer than the females.

From 271 to 280 days inclusive, 1,438 calves were born, of which 660 were males, while 778 were females. This represents 45.8 per cent of the males and 56.3 per cent of the females. In the group of 1,254 calves born from 281 to 290 days inclusive, 705, or 48.9 per cent, of the males and 549, or 39.7 per cent, of the females were included.

Beginning with the 271st day and up to and including the 280th day, more females than males were born each day. Beginning with the 281st day and up to and including the 295th day, more males than females were born each day with the exception of the 286th day when 58, or 4.2 per cent, of the females and 55, or 3.8 per cent, of the males were dropped.

In figure 1, it will be noticed that the line representing the females quite uniformly goes up one day earlier and down one day earlier than does the line representing the male calves.

In table 2 where the average gestation for the male and the female calves are segregated according to the age of the cow at calving in only two cases is the average gestation period longer for the female calves than for the males. These two cases are with nine-year olds and fourteen-year olds and for the latter there was only a total of seven calves.

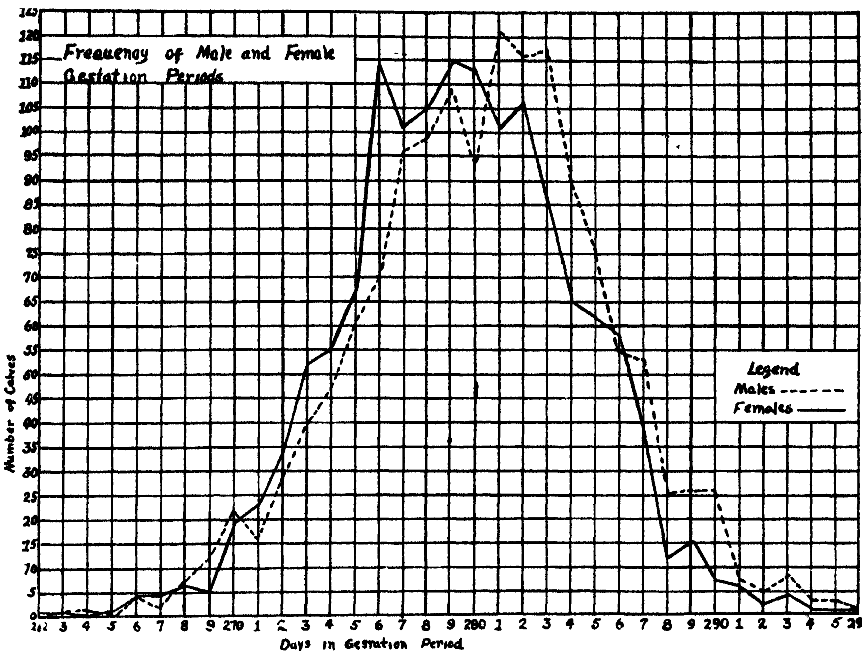

FIG. 1

TABLE 2

Average Length of Gestation Periods

AGE YEARS	NUMBER OF CALVES			AVERAGE LENGTH OF GESTATION		
	Males	Females	Total	Males	Females	Both Sexes
1	12	3	15	279.4	277.3	279.0
2	314	318	632	279.8	278.8	279.3
3	266	240	506	279.6	279.0	279.3
4	197	212	409	280.7	279.2	279.9
5	153	140	293	281.1	279.6	280.4
6	121	119	240	281.0	280.4	280.7
7	103	94	197	280.6	280.5	280.6
8	88	85	173	281.1	279.1	280.1
9	72	56	128	280.0	280.5	280.2
10	53	41	94	280.9	280.0	280.5
11	28	37	65	280.4	279.8	280.1
12	15	24	39	281.3	280.0	280.5
13	11	9	20	281.9	279.8	281.0
14	4	3	7	281.5	283.0	282.1
15	4	2	6	282.0	278.0	280.7
Totals	1,441	1,383	2,824	280.4 ± 0.090	279.4 ± 0.087	279.9 ± 0.063

Eight hundred twenty-five, or 57.3 per cent, of all male calves had a gestation period of 280 days or over, while there were but 677, or 49.0 per cent, of all females that were carried 280 days or more.

In considering the effect of the sex of the calf on the length of the gestation period, it is of interest to note that in studying the gestation period of twin calves, it was found that 24 male and male pairs had an average gestation of 276.5 days. Forty-two male and female pairs had an average gestation of 275.8 days, while 20 female and female pairs had an average gestation of 273.9 days. The number of twin births observed was only 86, but the results as given in table 3 are at least interesting and confirm the conclusion for single births—that males are carried longer than females.

TABLE 3

Distribution of Twin Births and Average Gestation Periods of Twins

GESTATION PERIOD DAYS	MALE AND MALE	MALE AND FEMALE	FEMALE AND FEMALE	TOTAL
265	1			1
267		1	2	3
268	1	2		3
269		2	2	4
270		2	3	5
271		2	2	4
272	3	3	1	7
273	3	2	1	6
274	1	2	1	4
275	1	2	1	4
276	2	5	1	8
277	1	5	1	7
278	2		1	3
279	2	3		5
280	2	6	1	9
281	1	2		3
282	1		1	2
283	2	1	2	5
285		1		1
287	1	1		2
Total	24	42	20	86
Average gestation	276.5 ± 0.687	275.8 ± 0.491	273.9 ± 0.762	275.5 ± 0.361

The average length of gestation periods for all twins was 275.5 days. This is 4.4 days less than the average gestation in the case of single births. The distribution of twin births is shown in table 3.

Table 2 gives the number of calves of each sex and of both sexes and the average gestation period of each sex, and of both sexes at the different ages of the dam. There seems to be a slight increase of approximately one and one-half days in the length of the gestation period from two-year olds to six-year olds.

While this slight increase may not be significant, it is interesting as indicating a possible tendency for the length of the gestation period to increase from the time a cow is a two-year old until she reaches maturity, after which there is a tendency for the length of the period to decrease.

Table 4 gives the number of calves and the average gestation of calves for each of 28 sires.

TABLE 4

Influence of Sire on Gestation Period

SIRE	NUMBER OF CALVES			AVERAGE LENGTH OF GESTATION		
	Male	Female	Total	Male	Female	Both Sexes
A	194	174	368	280.5	280.5	280.5
B	54	59	113	279.1	277.4	278.2
C	70	79	149	281.5	280.1	281.2
D	77	78	155	281.4	279.4	280.4
E	50	71	121	279.3	278.6	278.9
F	44	54	98	280.6	278.1	279.2
G	41	33	74	281.0	279.6	280.4
H	24	24	48	278.2	277.7	277.9
I	28	34	62	278.7	277.9	278.3
J	21	14	35	281.5	279.2	280.6
K	23	10	33	280.4	279.1	280.0
L	9	17	26	280.0	278.3	278.9
M	14	18	32	277.3	277.4	277.4
N	7	7	14	280.7	282.9	281.8
O	16	9	25	278.3	277.0	277.8
P	12	17	29	288.6	284.3	286.1
Q	13	11	24	282.3	282.1	282.2
R	9	8	17	279.0	280.5	279.7
S	14	6	20	280.3	278.2	279.7
T	12	14	26	277.5	276.5	277.0
U	64	66	130	279.5	278.1	278.8
V	38	23	60	279.0	280.3	279.5
W	58	51	109	279.5	279.2	279.4
X	19	23	42	277.2	279.4	278.3
Y	17	15	32	279.5	278.1	278.9
Z	23	10	33	280.9	281.4	281.1
A1	33	28	61	284.5	281.7	283.2
B1	9	17	26	279.7	279.7	279.7

In studying the gestation periods as segregated by sires, there appeared to be some that were quite uniformly high with a few periods that diverged widely. The gestation periods of the calves by some sires were uniformly short with a very few that diverged quite widely. The periods of others were quite uniformly average in length with a few long and a few short, while the calves from other sires seemed to have gestation periods quite uniformly and widely scattered over a considerable range.

TABLE 5

Gestation Periods of Individual Cows

COW NO.	AGE IN YEARS 2	3	4	5	6	7	8	9	10	11	12	13	Average
317	272	273	274	274	276		280						274.8
391		277		276	279	276	267	279					275.7
733	276	273	279	277	278	275	279	271	278				276.2
2204			278		271	276	282		272	279			276.3
2211					278	280	277		276	272	276		276.5
365		279	272	274	277	276	282						276.7
409		271	273	270	279	279	290						277.0
403	272	278	268	276	278	275	283			287			277.1
715		283	270	279	277	274	282						277.5
2213		280		274	282	283	276	274		275			277.7
2114	277	281	281		278	283	271						278.5
248				277			280	286	272	279	277	281	278.9
2084		280	278		277	276	288	275					279.0
2603	284	287	280	276	276	273							279.3
974	276		282	279	284		283	273					279.5
67B	280		279	287		278	287	277	274				279.9
642				273	281	282	279	284	278	—	282		279.9
2027		281	276	282	282		272	289					280.3
708	284	284	280	273	280	283							280.7
712	278		285	280	279	280	285	280	281				281.0
755	272	280	279	286		283	282	285					281.0
863		279	279	280	279		285	287					281.5
2263						287	283	283	286	277	281		282.8
2506		272		283	282	284	292	285					283.0
781				287	281		281	280	284	286			283.2
2070				279	285	287	294	280	283				284.7
2611	286	282	280	287	277	290							284.7
32B	276	290	288	284	285	287	284						284.9
2642	282	286	284	284	288	286							285.0

Table 5 gives the length of gestation for 29 cows that have produced six or more calves. It can be readily seen that individual cows vary widely in the length of gestation. The smallest variation was six days and this degree of uniformity is shown by but one cow. Two cows showed a variation of seven days. The extreme variation was 20 days and there were two cows that varied that much. The cow number 2642, with a variation of only six days, had a standard deviation of 1.9149 and a coefficient of variation of 0.677 per cent, while cow number 2506, with a variation of 20 days, had a standard deviation of 5.8878 and a coefficient of variation of 2.080 per cent.

Many of the cows showed quite uniform gestation periods while others showed widely divergent periods. Some had quite uniform periods with perhaps only one period that was quite different from the others. An example of this is cow number 2506. It will be seen that her two-year old period was for only 272 days. Her next shortest period was for 282 days. She has periods of 283, 284, 285 and another somewhat widely diverging one of 292 days.

Another example is cow number 409. Five of her gestation periods range from 271 to 279 days, while the sixth was for 290 days.

Cow number 317 showed a steady increase in length of gestation period from her first calf as a two-year old until her last calf as an eight-year old. In contrast to this cow, number 2603 showed a decrease in length of gestation period from her second calf as a three-year old until her last calf as a seven-year old. Of the 29 cows considered, 15 had an extreme variation of from 12 to 20 days inclusive, while 14 had an extreme variation of from six to eleven days inclusive.

Table 6 gives the records of eleven high producing cows. The producing ability of these cows is indicated by the fact that one of them holds three world's records, another holds two world's records and a third holds one world's record.

In addition to the records of these cows, table 6 gives the length of time these cows were carried in dam, the number of calves each has produced, and the average length of the gestation periods of their calves.

In collecting data for this study, it was noticed that cow number 440 with two world's records to her credit was carried in dam only 270 days. Data were collected on the remainder of the cows given in the table to ascertain if there were other cows of outstanding merit that were carried in dam for periods that might be considerably below the average.

Cow number 2831 holds three world's records. She was carried in dam only 272 days. This cow is a very large and rugged cow, weighing about 2,000 pounds before freshening. She has dropped four fine calves, the first as a junior two-year old, the second as a senior three-year old, the third as a junior four-year old and the fourth as a six-year old. She is now just

TABLE 6

The Length of Gestation Periods of High Producing Cows

EAR TAG	MILK LBS.	FAT LBS.	AGE		CARRIED IN DAM	GESTATIONS	
			Years	Mo.		No.	Ave.
2831	29,082	1,198	4	10	272	4	273.0
3305	28,100	920	2	2	282	1	276.0
2787	28,967	939	2	6	262	4	282.0
2664	30,760	948	6	4	279	5	281.0
2803	23,879	992	4	3	289	4	283.0
2818	24,282	892	5	8	276	2	273.0
2841	27,831	951	5	1	289	3	284.7
2868	25,392	905	4	5	273	3	282.0
2819	25,118	910	5	6	284	4	274.5
2904	30,366	924	3	5	282	3	277.7
440	23,250	932	3	5	270	4	279.5

seven years old and is strong and healthy in every way and carrying her fifth calf.

Cow number 440 was carried in dam only 270 days. She calved first as a junior two-year old, making a record that ranked fifth for that age. She calved again as a junior three-year old and at that age made a world's record for ten months' production, calved again as a junior four-year old and made a world's record in the ten months' division for that age also. This cow has been a regular breeder as evidenced by the fact that she met the calving requirements for the ten months' division.

Cow number 3305 holds the world's record for milk production as a junior two-year old and was carried in dam 282 days.

Cow number 2787 has the second highest record in the world for her age. She was carried in dam only 262 days. She has calved regularly and has apparently not suffered any bad effects from the short time she was carried in dam.

These eleven high producing cows varied in the time that they were carried in dam from 262 to 289 days, with an average of exactly 278 days, which is 1.4 days lower than the average of all females included in this investigation. They have carried their calves an average of 278.8 days, which is an increase of 0.8 of a day in the direction of the general average.

In table 6 is given the length of time eleven cows were carried in dam and also the number of calves produced by these cows and the average gestation periods for these calves.

Cow number 2831 was carried in dam 272 days and the average gestation periods for her four calves were 273.0 days. In contrast to this, cow number 2787 was carried in dam only 262 days, while the gestation periods of her four calves averaged 282.0 days. The shortest period for any of this cow's calves was 275 days.

Cows 2831 and 2818 were both carried for short gestation periods and the periods of their calves also tend to be short. Cows 2803 and 2841 were both carried for periods longer than average and their calves were also carried for periods longer than the average. There seems to be no correlation whatever in the case of the other seven cows between the length of time they were carried in dam and the length of time they carried their calves.

While the number of cases available is too small to warrant any definite conclusions as to the effect of heredity on the length of gestation period, the data indicate that this may be a factor.

SUMMARY

1. The average number of days of 2,824 gestation periods studied that terminated with single births was 279.9 ± 0.063. Twins were born in 86 cases following gestation periods averaging 275.5 days.
2. The length of gestation periods varied from 262 to 296 days. Almost 75 per cent of all calves were dropped from the 275th to the 285th days inclusive. Only 1.7 per cent were carried for periods shorter than 270 days and 2.7 per cent were carried for 290 days or longer.
3. Male calves were carried an average of one day longer than female calves.
4. An increase in the length of gestation periods of approximately one and one-half days was observed as the age of the dams increased from two to six years. After the age of six years was reached there seemed to be a tendency for the gestation periods to become slightly shorter.
5. The gestation periods of calves sired by some bulls were definitely higher or lower than the average, indicating by their uniform divergence from the mean the possibility of paternal influence.
6. The least variation in length of gestation observed for 29 cows that had each produced six or more calves was six days. The greatest variation was 20 days. The length of gestation periods of some cows were quite uniform, while others varied widely.
7. Eleven exceptionally high producing cows were carried in dam an average of 278 days, or about one and one-half days less than the average for all females. The periods varied from 262 to 289 days.
8. In comparing the gestation periods of the above cows with the gestation periods of the calves they produced, it was found that four of the eleven cases showed a decided tendency for the calves of a cow to have gestation periods similar in length to her own.

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THE EFFECT OF PASTEURIZATION ON THE BACTERIAL FLORA OF LOW COUNT MILK[1]

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INTRODUCTION

The application of improved methods of milk production on dairy farms during the past few years has resulted in a marked reduction of the bacterial contamination of market milk. In certain localities this reduction has been so pronounced that it is a rather common occurrence for the bulk of the milk received at pasteurizing plants to contain only a few thousand bacteria per cc.

As a result of the pasteurization of this low count milk, certain problems have developed which were not often encountered when milk of a greater bacterial content was being passed through the pasteurizer. It has been observed that such milk frequently does not coagulate even after a considerable period of time. It has also been noted that the absence of coagulation is oftentimes accompanied by the development of pronounced off flavors and odors. The nature of these off flavors and odors is usually such as to indicate changes in the nitrogenous constituents of the milk.

Past investigations of the bacterial content of raw milk and the influence of subsequent pasteurization have been made upon milk with a relatively high bacterial content. The milk studied frequently contained many millions of bacteria per cc. In addition, most of the investigational work pertaining to the numbers and types of organisms surviving pasteurization was carried out a number of years ago and at a time when high count milk was the rule and not the exception.

Ayers and Johnson (1) made a detailed study of the groups of bacteria developing in both raw and commercially pasteurized milk held at the same incubation temperatures. Their particular objects were to determine to what extent the lactic acid bacteria developed, and to compare the development of various groups of bacteria in pasteurized and raw milk. They divided the bacteria found in milk into three groups; namely, the

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alkali forming and inert, the lactic acid forming, and the peptonizing. As the result of their investigation, they make the following statement:

> "Commercially pasteurized milk always sours because of the development of lactic acid bacteria which on account of their high thermal death point survive pasteurization and perhaps in some cases because of subsequent infection with acid forming bacteria during cooling and bottling."

They found the relative proportions of the various groups of organisms to be approximately the same in efficiently pasteurized milk as in clean, raw milk. The alkali forming and inert types constituted the largest group. The next largest group was composed of the lactic acid bacteria, while the peptonizers were in the minority. They found that when both the efficiently pasteurized and the clean, raw milk were held at the same temperature the group relations changed, but the changes taking place were similar in each type of milk. At the time of souring the lactic acid bacteria constituted the largest group. Next in order were the alkali forming and inert types while the peptonizers were in the minority as initially. The majority of the samples of milk used by these investigators contained an initial bacterial content in excess of a million organisms per cc.

In a further study of the bacteria surviving pasteurization temperatures, Ayers and Johnson (2) obtained results somewhat different from those they had previously reported. Using milk of high bacterial content, they found that the percentage of the acid forming group was increased by pasteurization and that the percentages of all of the other groups were decreased. When pasteurization was below a temperature of 170°F for 30 minutes the bacterial flora was composed for the most part of organisms of the acid producing group. In the same paper, they state that when the best quality, or low count milk, was pasteurized, it was the only grade of milk showing a decrease in the percentage of acid-coagulating organisms.

It is to be remembered, however, that these investigators divided the acid forming bacteria into two groups, the acid coagulating, which produced sufficient acid to curdle the milk within 14 days, and the acid non-coagulating, which produced acid more slowly and did not curdle the milk in 14 days. Although the percentage of the acid coagulating group was reduced by pasteurization, the percentage of the total acid group was increased by this treatment. The grade of milk to which they referred contained an average bacterial content of 24,000 per cc. This seems to indicate that even different results might be obtained when milk with a much lower bacterial content is pasteurized. It is reasonable to assume that in high quality milk as indicated by a low bacterial count the bacterial flora of the normal cow's udder may influence, to a considerable extent, the percentage of the various bacterial groups surviving pasteurization.

These investigators noted a difference in the changes occurring in the various grades of milk following pasteurization. First, when a milk of fair quality was pasteurized at 145°F. for 30 minutes under laboratory conditions and held at room temperature the acid producing group might develop at once and overgrow all the other groups, resulting in the formation of acid and the production of a normal curd. Second, when a poor quality milk was pasteurized the peptonizing bacteria might grow rapidly at first along with the acid forming bacteria, which later overgrew them. Third, when a good grade of milk was pasteurized the peptonizing organisms might overgrow the acid producing group, thereby resulting in the peptonizing of the milk without the development of acid.

In a more recent paper in which the status of pasteurization is discussed Ayers (3) makes use of the experimental data gathered in the researches previously mentioned and applies them to the conditions existing some 13 to 15 years later. While he recognizes that variations in methods and conditions of production may influence considerably the bacterial group relations of an individual sample, he states that under average conditions milk which has been pasteurized at 145°F. for 30 minutes will sour instead of decompose. The explanation for this is that in milk pasteurized at the above temperature the majority of the viable organisms are of the kind which produce acid and as the milk is held these forms develop more rapidly than do the peptonizing forms. The average sanitary conditions pertaining to the production, handling and distribution of market milk, as they exist at the present time, or as they existed a few years ago, are hardly comparable to the sanitary conditions that prevailed 18 to 20 years ago. In view of this greatly changed condition it is questionable if the experimental results obtained under the average condition of 18 to 20 years ago can be applied at the present time.

There is, therefore, a decided need for information on this problem as it exists in certain regions normally producing a low count milk. Accordingly, the experiments reported in this paper were designed to give information on the types of bacteria surviving the pasteurization of low count milk, the changes occurring in the developing flora subsequent to pasteurization and the relation of this flora to the changes taking place in the milk.

PROCEDURE

The samples of milk used in this investigation represented milk produced near the city of Pullman, Washington. No attempt was made to supervise the milking process, although care was taken to select samples from the morning milk. These were collected as soon as possible after milking, and taken to the laboratory at once. The milk sample was then divided into two equal portions and one portion pasteurized in a flask at a temperature of 142.5° F. ± 0.5° for 30 minutes, after which it was closed immediately. Both raw and the pasteurized portions were divided again and a representative sample of each incubated at temperatures of 68°F. and 45°F., respectively. The sample under observation was examined for flavor daily and when an off flavor had developed so that the milk was unfit for consumption, it was considered spoiled.

In this study the bacteria contained within the milk were divided into three groups; the acid producing, the proteolytic, and a group including alkali forming and inert types. Differential and total bacterial counts were made on the raw and pasteurized portions at the time they were fresh and again at the time when spoilage was evident. These counts were determined by the use of Bacto-nutrient caseinate agar, a modification of the medium recommended by Ayers and Mudge (4). Prior to the sterilization of the medium a stock solution of indicator consisting of 0.4 gram of brom cresol purple, 7.4 cc. of N/10 NaOH and 92.6 cc. of distilled water was added to the agar at the rate of 8 cc.per liter. On plates poured with this medium the acid forming colonies appear yellow against a purple background, while the alkali forming colonies assume a purple color deeper than the surrounding medium. After these types have been enumerated the plate is flooded with dilute acetic acid. The proteolytic organisms are then distinguished by the clear halos surrounding the colonies. Those organisms that were not characterized by either acid forming, proteolytic or alkali forming properties were considered as inert types. Plate cultures were incubated at 37°C. for 48 hours.

Acidity and Cooledge score determinations were made on the raw and pasteurized portions of each sample at the time the samples were fresh and at the end of each 24-hour period throughout the incubation period.

PRESENTATION OF DATA

Bacterial Counts of Raw and Pasteurized Milk.

The initial bacterial counts of the 20 samples of milk used in this study ranged from 930 to 10,650 per cc. as determined by the use of caseinate agar plate cultures. Sixteen of these samples contained less than 5,000 per cc. The average bacterial count was 3,467 per cc. Pasteurization resulted in a reduction of the bacterial numbers ranging from 61.3 per cent to 99.95 per cent, with an average reduction of 89.1 per cent. The average number of organisms after pasteurization was 374 per cc.

Bacterial Groups Surviving Pasteurization and Developing in Raw and Pasteurized Milk Incubated at 68°F. and 45°F., respectively.

The bacterial groups in the raw and pasteurized samples were determined at the time the samples were fresh and again when the samples had deteriorated to such an extent as to be unfit for consumption. The average initial percentages of these groups in raw and pasteurized milk, the average percentages after incubation and the average length of the incubation period for the various types of samples are shown in table 1.

TABLE 1

Average percentages of bacterial groups in fresh and incubated raw and pasteurized milk

TYPE OF MILK	BACTERIA PER CC.	PER CENT ACID FORMING	PER CENT PROTEOLYTIC	PER CENT ALKALI FORMING AND INERT	HOURS OF INCUBATION
Fresh raw milk	3,467	26.17	0.76	73.07	
Fresh pasteurized milk	377	14.64	0.62	84.74	
Raw milk incubated at 68°F.	977,000,000	68.69	0.038	31.25	54
Pasteurized milk incubated at at 68°F.	358,000,000	22.51	23.74	53.76	103
Raw milk incubated at 45°F.*	164,700,000	62.15	11.29	25.56	137
Pasteurized milk incubated at 45°F.**	69,240,000	11.38	13.85	74.77	164

* Average of 18 samples.

** Average of 16 samples.

As the result of pasteurization the acid forming group of organisms decreased from 26.17 per cent to 14.6 per cent of the total flora. This decrease is within the limits of random sampling and, therefore, it is not of statistical significance. The ratio of the proteolytic group to the other groups of organisms remained practically unchanged. The alkali forming and inert group increased from 73.07 per cent in the raw to 84.74 per cent in the pasteurized milk. Although this group showed a considerable increase this difference cannot be considered statistically significant for the reason stated above.

A rather wide variation in the percentages of the acid forming bacteria existed among individual samples of both fresh raw and pasteurized milk. A similar conditions existed in the percentages of the alkali forming and inert group, while with the percentages of the proteolytic group this variation was not pronounced.

In each type of milk the proteolytic group constituted only a small proportion of the flora. Fifteen samples of the raw and 17 samples of the pasteurized milk contained less than 1.0 per cent of these types of bacteria. In no sample of either kind of milk did they exceed 6.0 per cent of the total flora. The percentage variations of the acid producing group and the alkali forming and inert group contained within the samples of each type of milk are shown in figures 1 and 2 respectively. The number of samples

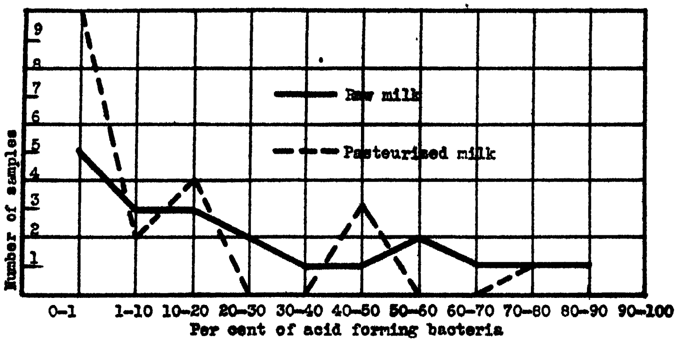

FIG. 1. COMPARISON OF THE INITIAL PERCENTAGES OF ACID FORMING BACTERIA IN SAMPLES OF LOW COUNT RAW AND PASTEURIZED MILK

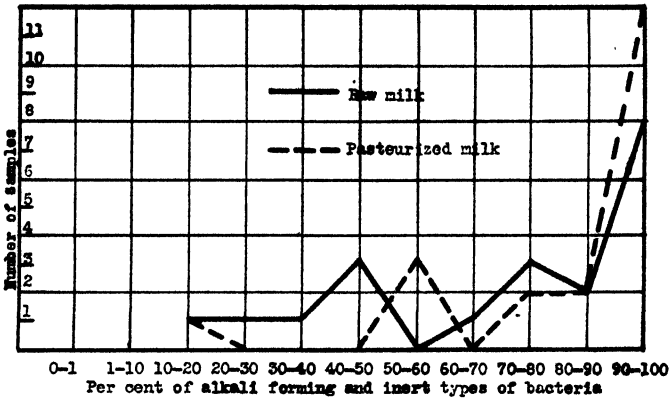

FIG. 2. COMPARISON OF THE INITIAL PERCENTAGES OF ALKALI FORMING AND INERT TYPES OF BACTERIA IN SAMPLES OF LOW COUNT RAW AND PASTEURIZED MILK

was plotted as ordinates and the percentage of the groups of organisms as abscissae, the ranges in percentage from 0–1, 1–10, 10–20, etc., being taken as points. It will be seen from the frequency curves thus obtained that pasteurization increased the number of samples in which the acid producing bacteria constituted 20 per cent or less of the total flora. In a like manner pasteurization increased the number of samples in which the alkali forming and inert group composed 70 per cent or more of the total flora.

Ayers and Johnson (2) grouped the milk with which they worked into three grades. The average bacterial content per cc. for each of these grades was as follows: A—32,000,000 (20 samples), B—3,500,000 (24 samples), and C—24,700 (12 samples). These investigators separated the acid producing bacteria into two groups; the acid coagulating and the acid non-coagulating. They also reported an alkali forming group and an inert group of bacteria. For the sake of comparison if the acid forming bacteria are combined into one group, and the alkali forming and inert groups combined in a like manner we find the group percentages as reported by them immediately before and after pasteurization at 145° F. for 30 minutes to be as follows:

GRADE		PER CENT ACID FORMING	PER CENT PROTEOLYTIC	PER CENT ALKALI FORMING AND INERT
A	Raw	46.88	17.31	35.78
	Past.	79.78	1.39	18.83
B	Raw	22.72	14.10	63.17
	Past.	66.76	29.23	3.59
C	Raw	40.70	12.81	46.46
	Past.	73.10	4.47	22.43

In each of the three grades of milk pasteurization resulted in an increase in the percentages of the acid forming group and a decrease in the percentages of the alkali forming and inert group. Opposite results were obtained in our experiment. Pasteurization resulted in a decrease in the acid forming group and an increase in the alkali producing and inert group, although these differences were not great enough to be of statistical significance.

A comparison of the final differential counts of the raw and pasteurized milk samples incubated at 68°F. show some significant differences in the development of the different groups of bacteria. The predominating group in the raw samples was composed of the acid producing types, while in the pasteurized samples the majority of the bacteria were of the alkali forming and inert types. A comparison of the percentages of the groups composing the total flora in the raw and pasteurized milk shows the acid producing group to average 68.69 per cent and 22.51 per cent respectively. The difference in percentages is so great that it assumes statistical significance. The pasteurized milk spoiled with a decided off flavor and in a few cases showed evidence of proteolysis. The acid producing types in the pasteurized milk remained the least abundant. The proportion of the proteolytic types was considerably greater in the pasteurized than in the raw samples, being increased from 0.038 per cent in the raw milk to 23.74 per cent in the pasteurized milk. The alkali forming and inert group was also considerably greater in the pasteurized than in the raw milk. Since in the pasteurized samples the acid producing group was in the minority, this group probably had an unimportant part in the spoiling of the milk even though the samples were incubated for a longer period than the raw milk samples.

The most striking variations in the percentage of the groups of bacteria developing in the raw and pasteurized milk samples incubated at 68°F.

occurred in the acid forming and the alkali producing and inert groups. The proteolytic group percentages showed no variation in the samples of the raw milk, but did show a considerable variation in the case of a number of samples of the pasteurized milk. None of the raw and only eight of the pasteurized milk samples contained proteolytic organisms in excess of 1.0 per cent of the total flora. Four of the samples of the pasteurized milk contained organisms of this group in sufficient numbers so that they constituted 80 per cent or more of the bacteria present. The proteolytic group in each of these four samples, immediately following pasteurization and prior to incubation, constituted less than 1.0 per cent of the flora. These organisms were able to overgrow an initial predominating flora composed almost entirely of alkali forming and inert types of bacteria. Frequency curves showing the variations in the percentages of the acid forming and the alkali producing and inert groups of bacteria in the samples of each type of milk are shown in figures 3 and 4 respectively.

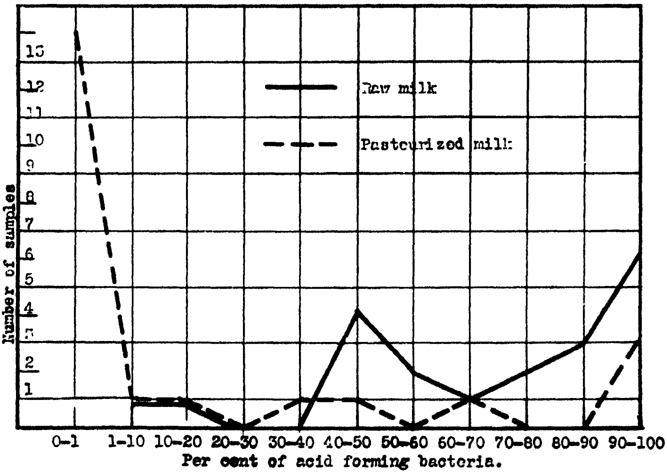

FIG. 3. COMPARISON OF THE PERCENTAGES OF ACID FORMING BACTERIA DEVELOPING IN SAMPLES OF LOW COUNT RAW AND PASTEURIZED MILK INCUBATED AT 68°F.

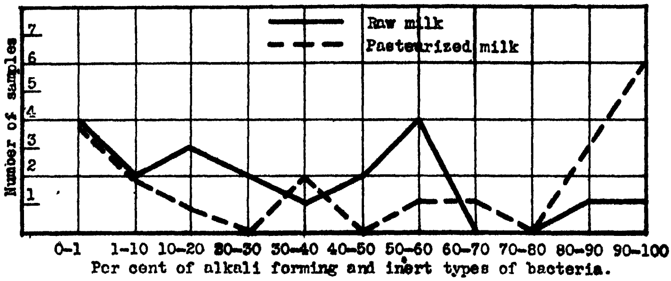

FIG. 4. COMPARISON OF THE PERCENTAGES OF ALKALI FORMING AND INERT BACTERIA DEVELOPING IN SAMPLES OF LOW COUNT RAW AND PASTEURIZED MILK INCUBATED AT 68°F.

From these curves, it will be seen that the acid forming group of organisms constituted more than 50 per cent of the bacterial flora in 14 of the raw milk samples as compared to four samples of the pasteurized milk containing like percentages of this group of organisms.

In 12 of the pasteurized milk samples these bacteria constituted less than 1.0 per cent of the organisms present. The alkali and inert group of bacteria composed 60 per cent or more of the flora in 10 of the pasteurized samples, whereas a like number were present in only two samples of the raw milk.

In the samples incubated at 45°F. the acid producing group predominated in the raw and the alkali forming and inert group predominated in the pasteurized milk. The acid producers in the raw and pasteurized milk averaged 62.15 per cent and 11.38 per cent respectively. Statistically this is a significant increase of the acid forming group in the raw milk over the

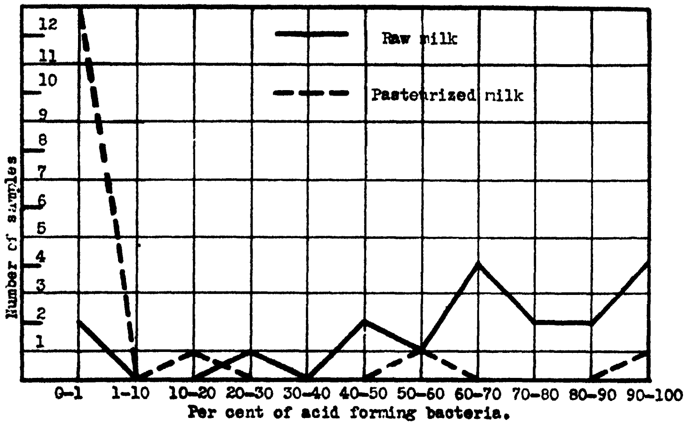

FIG. 5. COMPARISON OF THE PERCENTAGES OF ACID FORMING BACTERIA DEVELOPING IN SAMPLES OF LOW COUNT RAW AND PASTEURIZED MILK INCUBATED AT 45°F.

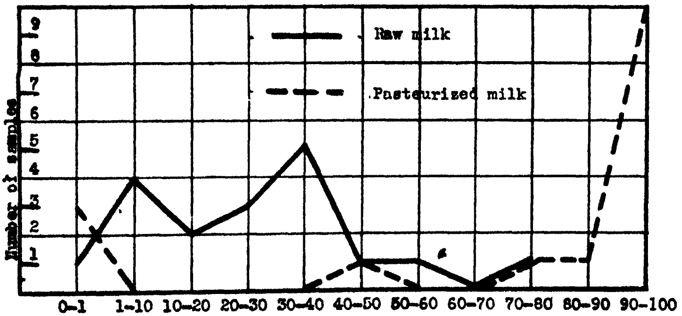

FIG. 6. COMPARISON OF THE PERCENTAGES OF ALKALI FORMING AND INERT BACTERIA DEVELOPING IN SAMPLES OF LOW COUNT RAW AND PASTEURIZED MILK INCUBATED AT 45°F.

acid forming group in the pasteurized milk. The percentage of proteolytic bacteria averaged 11.29 in the raw milk and 13.85 in the pasteurized milk. In the pasteurized milk the alkali forming and inert group predominated with an average of 74.77 per cent, while in the raw milk only 25.56 per cent of the flora was of this group. Statistically this is a significant increase. Although the low holding temperature had a decided retarding effect on the development of all bacteria, the raw milk eventually spoiled, due to the action of acid producing bacteria. The pasteurized milk, even though held 28 hours longer than the raw milk, did not sour but spoiled apparently due to the action of the proteolytic and the alkali forming and inert groups of bacteria.

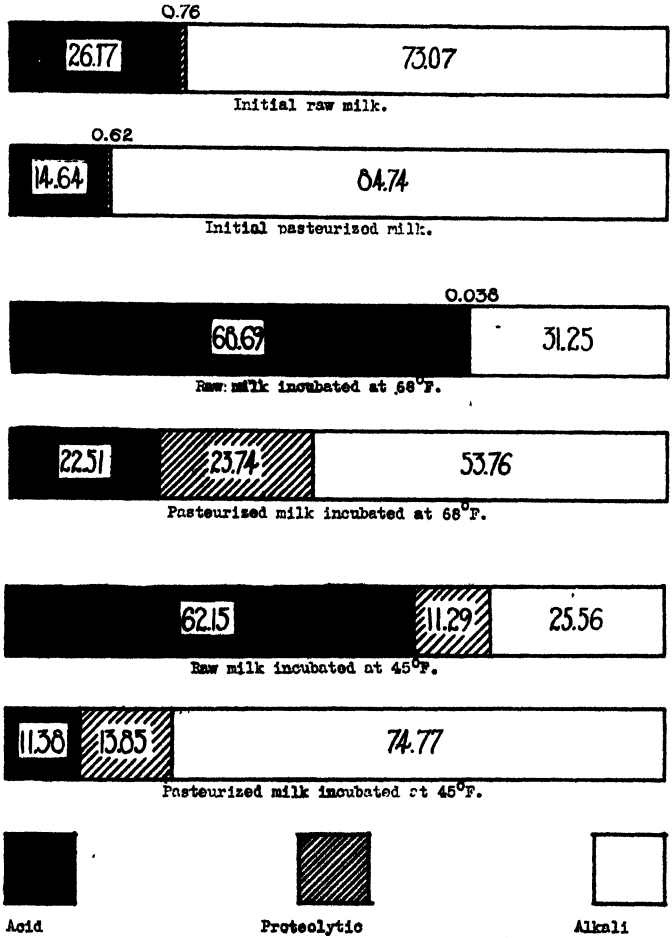

FIG. 7. COMPARATIVE PERCENTAGES OF BACTERIAL GROUPS DEVELOPING IN INCUBATED RAW AND PASTEURIZED MILK

The variations in the percentages of the acid forming group and the alkali producing and inert group of organisms in both raw and pasteurized samples incubated at 45°F. were very similar to those found in the samples incubated at 68°F. With both raw and pasteurized milk the proteolytic group of organisms constituted less than 10 per cent of the total flora in the majority of the samples. This group composed 70 per cent or more of the total flora in only two samples of each type of milk.

Frequency curves showing the variations in the percentages of the acid forming and the alkali forming and inert groups of bacteria in the samples of each type of milk are shown in figures 5 and 6 respectively. The acid producing group constituted 50 per cent or more of the bacterial flora in 13 of the 18 samples of raw milk while in 13 of the 16 samples of pasteurized milk less than 1.0 per cent of the flora was composed of this group of organisms. Organisms of the alkali forming and inert group of bacteria constituted less than 40 per cent of the flora in 15 of the 18 samples of raw milk. This same group of bacteria made up 70 per cent or more of the flora in 12 samples of the pasteurized milk.

The average percentages of the various groups of bacteria developing in raw and pasteurized milk at the two above incubation temperatures are shown graphically in figure 7.

The Cooledge Score of Raw and Pasteurized Milk Incubated at 68°F. and 45°F.

In the determination of the Cooledge score Bacto-Cooledge broth was used following the technique of Devereaux (5). The average scores for the 20 samples of each of the above series are shown in table 2.

TABLE 2
Average Cooledge scores of low count raw and pasteurized milk incubated at 68° F. and at 45° F.

TYPE OF MILK	NO. OF SAMPLES	0 HRS.	24 HRS.	48 HRS.	72 HRS.	96 HRS.
Raw milk at 68°F.	20	98	75	58		
Past. milk at 68°F.	20	99	95	84		
Raw milk at 45°F.	20	98	91	86	77	69
Past. milk at 45°F.	20	99	98	98	95	90

At both temperatures the decrease in score of the pasteurized samples was slower and did not reach as low a point as it did in the raw samples. Since this test is based upon the development of acid in the test medium, the results obtained in this experiment furnish additional evidence that the acid producing types were less active in the pasteurized than in the raw milk held at the same incubation temperature.

Acid Development in Raw and Pasteurized Milk Incubated at 68°F. and 45°F.

The acidity of the milk was calculated as the percentage of lactic acid. The results obtained are given in table 3.

TABLE 3

Average acidity of raw and pasteurized milk incubated at 68°F. and at 45°F.

TYPE OF MILK	NO. OF SAMPLES	0 HRS. PER CENT ACID	24 HRS. PER CENT ACID	48 HRS. PER CENT ACID	72 HRS. PER CENT ACID	96 HRS. PER CENT ACID
Raw milk at 68°F.	20	0.18	0.19	0.51		
Past. milk at 68°F.	20	0.18	0.19	0.20	0.21	0.23
Raw milk at 45°F.	20	0.18	0.18	0.19	0.22	0.27
Past. milk at 45°F.	20	0.18	0.18	0.18	0.18	0.19

From the results of these determinations, it is very evident that the acid producing flora developed more rapidly in the raw than in the pasteurized milk. This is especially striking in the milk incubated at 68°F. After 48 hours of incubation, 13 of the raw milk samples had reached an acidity in excess of 0.5 per cent, whereas the highest acidity recorded for any sample of the pasteurized milk at the end of 96 hours' incubation was 0.43 per cent. The acid producing bacteria which were found to predominate in four samples of the pasteurized milk apparently were of the non-coagulating type. Even after a period of 96 hours, the acidity of the milk incubated at 45°F. had increased but little. This is especially true of the pasteurized samples. At the end of this time, seven of the raw milk samples had reached an acidity greater than 0.3 per cent, ranging from 0.31 per cent to 0.57 per cent, while none of the pasteurized milk samples showed an acidity of more than 0.23 per cent. These results indicate that the acid forming bacteria which develop in raw milk at 45°F. consist for the most part of the non-coagulating type. Since an increase in acidity is due to an increase in the numbers of acid forming bacteria, there was a greater preponderance of these forms of organisms in the raw than in the pasteurized milk at the time of spoilage. These results substantiate the differential count and the Cooledge score determinations.

SUMMARY AND CONCLUSIONS

Plate counts were made on 20 samples of milk before and after pasteurization. The average count before pasteurization was 3,467 per cc. and after pasteurization 377 per cc.

The bacterial flora of the raw and pasteurized milk was divided into three principal groups: the acid producing, the proteolytic and the alkali forming and inert. In the raw milk the acid producers composed 26.17

per cent, the proteolytic 0.76 per cent, and the alkali forming and inert 73.07 per cent of the bacterial flora present. In the freshly pasteurized milk the acid producers composed 14.64 per cent, the proteolytic group 0.62 per cent and the alkali forming and inert group 84.74 per cent of the organism present.

Raw milk was incubated at 68°F. until it spoiled. The acid group increased from 26.17 per cent to 68.69 per cent, and the proteolytic group decreased from 0.76 per cent to 0.038 per cent. The alkali forming and inert group decreased from 73.09 per cent to 31.25 per cent. When the raw milk was incubated at 45°F. until it spoiled the acid forming group increased from 26.17 per cent to 62.15 per cent. The proteolytic group increased from 0.76 per cent to 11.29 per cent and the alkali forming and inert group decreased from 73.07 per cent to 25.56 per cent.

Pasteurized milk was incubated at 68°F. until it spoiled. The acid producers increased from 14.64 per cent to 22.51 per cent. The proteolytic group increased from 0.62 per cent to 23.74 per cent and the alkali forming and inert group decreased from 84.74 per cent to 53.76 per cent. When incubated at 45°F. the acid group decreased from 14.64 per cent to 11.38 per cent. The proteolytic group increased from 0.62 per cent to 13.85 per cent and the alkali forming and inert group decreased from 84.74 per cent to 74.77.

Cooledge score and titratable acid determinations were made on both raw and pasteurized samples at the beginning of the experiment and every 24 hours until the milk spoiled. Both of these tests furnished additional evidence relative to the groups of organisms responsible for the changes occurring in incubated raw and pasteurized milk.

These results indicate that when milk of low initial bacterial content is pasteurized the acid producing bacteria are not of major importance in the spoilage of the milk. This may account in part for the trouble occurring in certain localities where low count milk is being pasteurized. Such milk often spoils with a decided off flavor and odor, but without the usual evidence of souring. From the results of this investigation it seems likely that this trouble may be attributed to the action of the proteolytic and the alkali forming and inert types of bacteria. This may especially be true when contamination subsequent to the pasteurizing process is eliminated.

The evidence reported in this paper bearing on the groups of bacteria surviving pasteurization and causing the subsequent changes in milk does not fully agree with the results obtained by other investigators. The conclusions reached by these investigators were based on experiments with milk of relatively high bacterial content. Their milk samples frequently contained many millions of bacteria per cc. while in our study a low count milk was used.

In this investigation, it is recognized that a sufficient number of samples have not been studied to warrant the formulation of definite conclusions. It is felt, however, that the observations recorded in this paper are indicative of the bacteriological changes which result when a very low count milk is pasteurized under commercial conditions.

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THE FOOD VALUE OF FROZEN EVAPORATED MILK

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Very little work has been done to determine whether or not the freezing of milk changes its food value. It is important to determine this point as many people, including nutritionists, have believed frozen milk to be deleterious to health. The thought that something has gone wrong has probably resulted from the fact that freezing does cause a change in the appearance of the milk. There is frequent opportunity for evaporated milk to become frozen, particularly that which is used in arctic regions.

Anderson and Pierce (1) in a recent article state that the precipitation of milk protein from raw and sterile skim milk takes place after 2 months' storage at + 10° F. and after 3 months' storage at – 14° F. They also state that some slight chemical changes must take place in the milk stored as above inasmuch as they were able to detect an increase of amino-nitrogen. Smith (2), in 1922, studied the vitamins in ice cream and found that for vitamin A "no noteworthy alteration in its potency was caused by pasteurization and freezing." Normal growth in rats was induced by the addition of one gram of ice cream (10% fat) per day to the diet, and xerophthalmia was cured by amounts as small as 0.25 grams containing but 25 milligrams of butter fat. Freezing has no effect on vitamin B, and ice cream made from pasteurized products was found to contain very little vitamin C.

The procedure followed in the experiment herein described consisted in feeding separate sets of albino rats on evaporated milk which had been stored in a normal manner and evaporated milk which had been frozen in cold storage and observing the rate of growth of each set of rats.

All evaporated milk used was of the date of May 8, 1930. Part was stored under normal storage conditions and part was stored 5 months and 20 days at – 5° F., followed by 30 days' storage at normal temperatures.

The albino rats used were 27 days old at the start of the experiment and weighed about 40 grams each. Twenty-two rats were fed the frozen-thawed evaporated milk and twenty were fed the normal evaporated milk. All rats were on a milk diet entirely, being given all the milk which they would consume. All of the milk was diluted with distilled water to the consistency of ordinary market milk before feeding (1 : 1). Osborne and Mendel (3) in their experiments reached the conclusion that rats required at least 16 grams of ordinary milk daily for normal growth. It was found in this experiment that the rats would consume usually more than 16 grams of evaporated milk daily, equivalent to more than 32 grams of ordinary milk.

At the start of the experiment all rats developed "scours" as a result of being placed on an exclusively liquid diet. During this period no difference could be seen between rats on the different milk, and in two weeks both sets of rats had adjusted their systems to the liquid diet.

Tables 1 and 2 give the gain in weight of the rats on the different milks over a period of 12 weeks, and the averages. The figures in the column on the extreme right show the average amount of milk consumed daily by each rat.

TABLE 1

Weight of rats in grams fed evaporated milk not frozen

RAT NO.	AT START	1ST WEEK	3RD WEEK	6TH WEEK	9TH WEEK	12TH WEEK	AV. MILK (DILUTED) CONSUMED PER DAY
							grams
1	36	52	61	82	105	120	36.0
2	29	35	44	Died			.
3	31	41	65	74	66	71	30.4
4	37	44	61	75	93	106	36.0
5	40	50	54	44	Died		..
6	31	43	55	59	59	50	28.7
7	44	56	67	82	97	108	36.0
8	40	49	70	71	92	108	36.0
9	38	50	59	68	82	95	31.5
10	37	50	62	78	96	114	36.0
11	42	45	63	70	81	109	31.0
12	49	46	55	61	75	92	30.0
13	50	49	55	63	80	113	31.0
14	41	46	62	68	84	109	31.0
15	42	40	56	73	80	92	30.0
16	38	40	52	61	80	104	31.0
17	40	44	54	64	81	105	30.0
18	44	40	47	60	74	101	31.0
19	40	42	55	65	76	94	31.0
20	41	36	49	59	67	84	27.0
Average gain		5.4	17.8	27.7	42.0	57.1	

SUMMARY

The growth of young rats on normal evaporated milk and on frozen-thawed evaporated milk was determined.

The data show that during the 12 weeks' period of the experiment the rats fed on normal evaporated milk made an average gain in weight of 57.1 grams on an average daily food consumption of 32.1 grams (in terms of ordinary milk). In comparison, the rats fed on frozen-thawed evaporated milk made an average gain of 58.2 grams on an average daily food consumption of 33 grams.

It will be noted that the rats fed on frozen-thawed evaporated milk consistently made the larger gain in weight for any given period. However the

TABLE 2

Weight of rats in grams fed frozen-thawed evaporated milk

RAT NO.	AT START	1ST WEEK	3RD WEEK	6TH WEEK	9TH WEEK	12TH WEEK	AV. MILK (DILUTED) CONSUMED PER DAY
							grams
21	40	48	61	75	91	105	36.0
22	43	51	57	63	96	110	32.2
23	38	48	67	75	90	105	36.0
24	43	51	70	75	90	103	36.0
25	42	54	72	76	90	102	36.0
26	41	56	73	86	96	109	36.0
27	43	53	68	77	89	101	36.0
28	42	56	69	79	96	106	36.0
29	41	53	71	87	103	112	36.0
30	43	57	69	85	103	114	36.0
30A	44	54	68	70	80	83	33.8
31	48	44	54	66	82	104	31.0
32	43	39	51	62	83	104	31.0
33	43	39	49	61	76	91	31.0
34	38	40	50	60	76	92	31.0
35	40	38	47	54	67	90	30.0
36	42	43	47	55	65	64	30.0
37	47	42	52	63	75	90	31.0
38	41	41	55	71	89	105	31.0
39	45	43	52	60	76	94	31.0
40	39	38	50	62	77	100	31.0
41	41	40	48	64	75	91	29.0
Average gain		6.1	18.4	28.4	44.1	58.2	

difference between the two sets of rats is small, amounting to only 1.1 grams at the end of twelve weeks and is to be accounted for by the fact that the rats fed on frozen-thawed evaporated milk consumed on an average of 0.9 grams of milk more per day.

Based upon the growth data and upon the fact that all rats were in good physical condition at the end of the experiment, we can conclude that none of the food value of evaporated milk is destroyed by freezing, and neither is there produced any substance deleterious to health.

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THE CHEMISTRY OF THE BLOOD OF DAIRY COWS BEFORE AND AFTER PARTURITION AND ITS RELATION TO MILK FEVER

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INTRODUCTION

The disease milk fever has been known to dairymen for over 100 years. Since Schmidt's well known discovery in 1897 the treatment of this disease has been quite simple, but until recently, veterinarians have had only numerous and somewhat vague theories as to the cause of the disease. However, recent biochemical work has given us some definite information on the nature of milk fever.

The various phases of this disease have been covered quite thoroughly in recent reviews by Greig (11), Harding (12), Little and Wright (19), and Hayden and Scholl (14). The more recent theories on the cause of milk fever center around ideas of disturbances in metabolism or internal secretions, which may occur at the onset of lactation.

The hypoglycemia theory has been the subject of considerable investigation. It was started by the observation of the similarity of the symptoms of milk fever to those of insulin hypoglycemia. However, Hayden (13) and Fish (4) have investigated this question quite thoroughly and have extensive data showing that in milk fever there is a hyperglycemia rather than a hypoglycemia. They found that before inflation for milk fever the blood sugar was above normal and that it rose after inflation, later coming back towards normal. Lactose, as well as glucose, was found in the blood and in the urine after inflation.

Recent work on milk fever has focused attention on the blood Ca and blood P, particularly the Ca. Little and Wright (18) reported blood Ca determinations on 12 cows affected with milk fever. They found decreases below normal of 20 to 30 per cent in mild cases, and up to 60 per cent in severe ones. They also stated that 30 to 40 cc. of 10 per cent $CaCl_2$ solution injected intravenously or intramuscularly had a beneficial effect. Dryerre and Greig (2) advanced as a working hypothesis the idea of a parathyroid deficiency being a factor in this disease. Greig (11) gives data on 82 milk fever cases in which the average serum Ca was 5.13 mg. per 100 cc. while the minimum and maximum values were 3.00 and 7.76 respectively. It was found that udder inflation in milk fever cases caused a rise in serum Ca,

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which continued on above the normal and then returned to normal, the maximum occurring at 3 to 5 days after inflation. Greig suggested the injection of calcium gluconate solution as a treatment, and he (10) reported 32 cases, which were given this treatment, 23 of which recovered rapidly and completely as the result of one injection. The other 9 cases were given a second injection or the udder was inflated. Sjollema (24) reported low values for both Ca and P of the serum in 40 milk fever cases. He recommended injection of 300 to 400 cc. of 10 per cent $CaCl_2$ solution, and got reports of very good results from veterinarians who used this treatment. Fish (5) reported the following values for 25 milk fever cases: Ca, average 3.31 mg. per 100 cc., minimum and maximum, 1.75 and 6.00 respectively; P, average 2.39 mg. per 100 cc., minimum and maximum, less than 1 and 4.25 respectively. He found that after inflation in milk fever cases, the P returns to normal and goes on above normal in 6 to 8 hours, while on the average the Ca was still below normal after 24 to 36 hours.

Harding (12) has advanced the idea of anhydremia being present in milk fever. He believes that shock resulting from anhydremia could be responsible for all of the symptoms of milk fever. However, he admits that this theory does not explain satisfactorily the low blood Ca and P values which occur.

Although considerable work has been done on the chemistry of the blood in cases of milk fever, little has been reported on the changes in the blood, if any, that take place in normal parturitions. Dryerre and Greig (3) report data on two cows calving normally, which indicate a slight fall in serum Ca at the so-called milk fever period. Palmer, Cunningham and Eckles (21) found that normal parturition caused a decrease in inorganic P. They have also presented considerable data on the normal variations in the P of the blood of dairy cows. They found quite marked day to day variations, even when the blood was drawn under apparently identical conditions. Palmer and Eckles (22) report data on the normal variations in serum Ca.

It was considered that a study of the blood variations in case of normal calvings might reveal more definite knowledge as to just what factors are responsible for milk fever, and why some cows have it and other cows calving under very similar conditions do not. We do not know whether the disturbances that bring on milk fever are present in all parturitions, and only show their effect when they become acute, or whether milk fever is due to some peculiar or unusual disturbance. The investigation which is being reported was carried out with these ideas in mind.

INVESTIGATIONAL

Analyses were made of blood samples taken before and after calving from cows in the dairy herd of the Wisconsin Experiment Station. At first two samples only were taken, in most cases, one before calving and one after. Usually the samples were taken within a day of the time of calving. The samples were drawn from the jugular vein and were analyzed for total serum Ca, inorganic serum P, total sugar, and in a few cases, for non-fermentable reducing substance. Ca was determined by the Clark and Collip (1) method and P by the Fiske and Subbarow (6) method. With cow's blood the serum does not separate from the clot to any extent when it is cold, and for this reason the samples were set in the incubator for about an hour after they had clotted. It was found that the results on samples so treated checked very well with those on samples that were centrifuged immediately after drawing before the blood had clotted firmly. Total sugar and non-fermentable reducing substances were determined by the Folin and Svedberg (7) method. For sugar determinations blood samples of 10 to 20 cc. were collected in test-tubes containing 7 drops of 6.25 per cent potassium oxalate solution dried on the inside.

A summary of the data obtained in this preliminary work is given in tables 1 and 2. It will be seen that the values obtained for Ca and P vary considerably around calving time, but the variations are not consistent. In some cases these values are higher after calving, and in about an equal number of cases they are lower after calving. These data suggest that more significant results might be secured if more than two blood samples were taken for each individual case. Therefore, in continuing this work it was decided to take samples several days before calving and several days after, as well as samples very near the time of calving. The samples were analyzed for total Ca and inorganic P as before. Sugar determinations were discontinued as the blood sugar level is apparently not an important factor in the etiology of milk fever. In addition to Ca and P, the blood proteins; albumin, globulin, and hemoglobin, were determined. This was done to determine if there is any evidence of anhydremia in normal calvings, or in milk fever, as suggested by Harding (12). It was also considered interesting to see if the large amount of globulin secreted in the colostrum milk would affect the level of globulin in the blood.

The serum albumin and globulin were determined by the Greenberg (9) method. The results were calculated on the basis of the tyrosine equivalents of these proteins given by Greenberg for human blood. The hemoglobin was determined by the Newcomer (20) method using a Bausch and Lomb filter as a standard. Oxalated blood was used for all of the protein determinations. The data on individual cases for which blood proteins and total Ca and inorganic P were determined are given in table 3.

Later the protein determinations were discontinued and plasma phosphatase activity was determined. This enzyme was discovered in bone by Robison in 1923 and there is evidence that it plays an important rôle in bone formation. It is also found in other tissues. Kay (17) found that

TABLE 1

Average blood Ca, P and sugar values for cows before and after calving

Grouped according to the times the blood samples were taken

BLOOD CONSTITUENT	TIMES		NO.	BEFORE CALVING	AFTER CALVING	RISE AFTER CALVING			FALL AFTER CALVING		
	Before calving	After calving				No.	Ave.	Max.	No.	Ave.	Max.
Tot. sugar mg. per 100 cc. of blood	Over 10 hrs.	Over 10 hrs.	3	53.5	48.2	1	1.8		2	8.8	16.1
	Over 10 hrs.	10 hrs. or less	3	81.2	75.4	1	5.6		2	11.1	13.0
	10 hrs. or less	Over 10 hrs.	3	54.7	49.6	0			3	4.96	7.9
	10 hrs. or less	10 hrs. or less	4	68.2	72.2	2	16.9	22.9	2	8.9	13.8
Totals			13	64.7	62.2	4	10.3	22.9	9	8.03	16.1
	Before calving	After calving									
Ca. mg. per 100 cc. serum	Over 10 hrs.	Over 10 hrs.	3	10.5	10.4	1	0.2		1	0.6	
	Over 10 hrs.	10 hrs. or less	3	9.4	10.4	2	2.1	3.8*	1	1.5	
	10 hrs. or less	Over 10 hrs.	5	9.4	9.9	5	0.5	0.8	0		
	10 hrs. or less	10 hrs. or less	7	9.6	9.5	3	0.6	1.3	4	0.8	1.5
Totals			18	9.68	9.89	11	0.78	3.8*	6	0.81	1.5
	Before calving	After calving									
P. mg. per 100 cc. serum	Over 10 hrs.	Over 10 hrs.	3	7.41	5.96	1	0.23		2	2.32	2.66
	Over 10 hrs.	10 hrs. or less	3	5.00	5.59	2	1.10	1.99	1	0.43	
	10 hrs. or less	Over 10 hrs.	5	5.60	6.31	2	2.54	2.81	3	0.51	0.71
	10 hrs. or less	10 hrs. or less	7	5.29	5.88	5	1.30	2.54	2	0.62	1.71
Totals			18	5.67	5.97	10	1.40	2.81	8	0.98	2.66

* This figure may be questioned as the blood sample was frozen and hemolyzed considerably.

TABLE 2

Average blood Ca, P, and sugar values

Where samples were taken at two different times after calving

CONSTITUENT	NO. OF CASES	LESS THAN 10 HRS. AFTER	OVER 10 HRS. AFTER	RISE			FALL		
				No.	Ave.	Range	No.	Ave.	Range
Tot. Sugar mg. per 100 cc. of blood	4	91.2	59.9	0			4	31.3	3.2–70.9
Ca mg. per 100 cc. of serum	5	9.4	10.1	4	1.2	0.6 –2.1	1	1.2	
P mg. per 100 cc. of serum	5	5.42	6.01	4	1.48	0.83–2.63			

the plasma phosphatase is high in generalized bone diseases such as rickets and osteomalacia. He considers that the phosphatase in the blood plasma is present largely as the result of a leakage or slow diffusion from other tissues. Various workers have shown that high producing dairy cows frequently go into negative Ca balances for considerable lengths of time. In such cases the Ca is evidently being withdrawn from the bones. Since a high plasma phosphatase is present in rickets and in osteomalacia, where bone decalification is going on, it was thought that some significant variations might be found in the amount of this enzyme in the blood of dairy cows before and after calving, and in cases of milk fever. The phosphatase was determined by the method of Kay (16). The data on individual cases for which plasma phosphatase and inorganic Ca and P were determined are shown in table 4.

Phosphatase activity was also determined in the milk as well as the blood plasma of a few cows. Some low phosphatase values were found in the plasma of some of the heavily producing cows and this suggested that there might be some elimination of the enzyme from the blood through the milk. The phosphatase in the milk was determined in the same way as in the plasma (except for a slight modification) so that the data on the activities of the two fluids may be considered comparable. The data on this work are given in table 5. These data show that the phosphatase activity of the milk is usually greater than that of the plasma, particularly in cows near the end of lactation. The amount in the milk is roughly, inversely proportional to the amount of milk being secreted. This suggests that the enzyme may occur in the tissues of the udder, and it is possible that it plays a rôle in the phosphorus metabolism involved in milk secretion. It is at least

TABLE 3

Analyses of normal cows' blood before and after calving for blood proteins and total Ca and inorganic P

Hemoglobin = % of whole blood; albumin and globulin = % of serum; Ca and P = mg. per 100 cc. serum

COW NO. AND LACTATION		BEFORE CALVING		AFTER CALVING	
No. 1	Time	11 days	2 hrs.	14 hrs.	7 days
1st Lact.	Hemoglobin				
	Albumin	3.59	3.94	3.39	3.52
	Globulin	3.54	2.66	2.98	3.02
	Ca	11.5	11.7	11.0	10.7
	P	5.40	3.86	5.78	6.34
No. 2	Time	12 days		2½ hrs.	9 days
3rd Lact.	Hemoglobin			12.35	11.05
	Albumin	3.70		5.28	4.86
	Globulin	2.50		3.83	2.49
	Ca	10.8		10.6	11.8
	P	5.00		3.44	4.12
No. 4	Time	5 days	8½ hrs.	12½ hrs.	5 days
1st Lact.	Hemoglobin		8.80	8.08	8.30
	Albumin	3.62	4.58	4.38	3.42
	Globulin	1.76	2.23	2.22	2.12
	Ca	12.1		9.6	10.8
	P	5.28		8.42	5.74
No. 5	Time	7 days		3½ hrs.	7 days
1st Lact.	Hemoglobin	7.63		11.49	9.92
	Albumin	3.88		4.42	4.86
	Globulin	2.12		3.48	3.64
	Ca	9.8		9.8	9.5
	P	6.68		4.98	5.02
No. 6	Time	14 days		1 hr.	7 days
1st Lact.	Hemoglobin	9.92		12.35	10.75
	Albumin	4.25		4.28	3.80
	Globulin	3.12		2.81	3.06
	Ca	10.6		10.1	10.9
	P	5.46		3.70	6.72
No. 7	Time	10 days		2 hrs.	9 days
4th Lact.	Hemoglobin	11.83		11.16	10.00
	Albumin	3.64		3.74	3.86
	Globulin	3.22		3.31	3.96
	Ca	11.1		10.4	11.2
	P	6.52		2.84	5.12
No. 8	Time	6 days		2½ hrs.	6 days
1st Lact.	Hemoglobin	12.48		12.48	10.00
	Albumin	3.98		4.15	3.55
	Globulin	2.74		3.11	3.64
	Ca	10.4		10.4	10.4
	P	5.10		5.76	5.92
No. 9	Time	14 days		1½ hrs.	7 days
1st Lact.	Hemoglobin	9.68		10.75	8.47
	Albumin	4.15		4.02	4.02
	Globulin	2.40		2.42	2.72
	Ca	10.9		9.9	9.6
	P	5.22		4.04	6.22

TABLE 4

Analyses of normal cows' blood before and after calving for plasma phosphates and inorganic P and total Ca

Phosphatase = units of phosphatase as defined by Kay, 1930. Ca and P = mg. per 100 cc. of serum

COW NO. AND LACTATION		BEFORE CALVING						AFTER CALVING			
No. 12 3rd Lact.	Time				22 days	2 days		11 hrs.	5 days	8 days	22 days
	P'tase				.086	.195		.200	.179		.106
	P				6.06	7.04		8.04		4.32	5.80
	Ca				9.8	9.6		8.9		10.2	10.3
No. 13 1st. Lact.	Time					2 days		8 hrs.	5 days	9 days	16 days
	P'tase					.100		.106	.101	.172	.200
	P					6.81		7.88	2.80	5.62	
	Ca					10.9		10.9	10.7	11.1	
No. 14 4th Lact.	Time					12 days		11 hrs.	8 days	18 days	
	P'tase					.0698		.163	.145	.090	
	P					9.12		4.40	5.72		
	Ca					10.2		9.0	10.0		
No. 15 2nd Lact.	Time					5 days		4 hrs.	9 days		
	P'tase					.086		.238	.178		
	P					4.80		6.58	5.74		
	Ca					10.6		8.7	10.6		
No. 16 2nd Lact.	Time	16 days	8 days	6 days	4 days	2 days	2½ hrs.	6 hrs.	1 day	3 days	6 days
	P'tase	.168	.110					.180			.097
	P		6.26	6.14	6.52	5.52	4.20	5.72	4.52	4.93	4.92
	Ca.		11.1	10.8	11.8	10.9	10.6	10.3	10.4	10.2	10.8
No. 18 2nd Lact.	Time					35 days	15 hrs.	1 day	3 days	5 days	10 days
	P'tase					.225	.114	.097	.095		.085
	P						5.24	4.30	4.08	3.22	6.60
	Ca						9.4	8.3	9.2	8.5	9.2

TABLE 4—(*Continued*)

COW NO. AND LACTATION		BEFORE CALVING						AFTER CALVING			
No. 20 2nd Lact.	Time	40 days	9 days	7 days	5 days	3 days	2 hrs.	7 hrs.	1 day	4 days	19 days
	P'tase	.167	.103			.106		.193			.073
	P		5.44	4.96	4.88	5.40	5.42	6.82	6.08	5.06	6.42
	Ca		10.0	9.5	10.15	9.8	9.5	9.8	8.8	9.1	9.2
No. 21 1st Lact.	Time	31 days	11 days	9 days	6 days	4 days	20½ hrs.	1 day	3 days	14 days	
	P'tase	.189	.150					.168		.074	
	P		6.22	5.72	5.58	5.78	4.52	3.94	5.72	6.28	
	Ca		9.3	9.7	9.7	10.1	9.8	8.4	9.8	10.1	
No. 22 8th Lact.	Time	55 days	8 days	6 days	2 days	5½ hrs.		4 hrs.	2 days	5 days	9 days
	P'tase	.162	.059					.094			.099
	P			4.70	4.18	4.88		3.90	4.14	5.92	4.42
	Ca			10.4	10.1	9.2		9.5	8.3	8.8	9.3
No. 24 3rd Lact.	Time			46 days	9 days	2 days		8 hrs.	1 day	3 days	7 days
	P'tase			.214	.089				.228		.154
	P					5.54		6.86	6.10	6.10	5.54
	Ca					10.6		9.6	9.9	9.7	10.5
No. 25 1st Lact.	Time		42 days	11 days	2 days	16 hrs.		9 hrs.	1 day	4 days	11 days
	P'tase		.170	.110				.172			
	P			4.28	4.30	4.26		5.74	4.44	5.32	5.96
	Ca			9.4	10.5	9.0		10.1	8.95	10.2	10.5
No. 11 4th Lact.	Time					7 days		1 day	7 days		
	P'tase					.085		.095	.084		
No. 19	Time					29 days		1 day			
	P'tase					.182		.128			

TABLE 5

Phosphatase values obtained for the blood plasma and the milk of dairy cows

	PHOSPHATASE UNITS		
	Plasma		Milk
Cows near the end of lactation	1	.162	.838
	2	.094	.990
	3	.068	.637
Average		.108	.8217
Cows milking heavily	1	.090	.141
	2	.075	.083
	3	.097	.046
Average		.0873	.090

evident that the presence of this enzyme in the milk is not due entirely to simple diffusion from the blood plasma.

In addition to the work on the blood of cows in cases of normal calving, analyses were made on samples from a number of typical cases of milk fever and one case which was apparently on the verge of having the disease but did not come down with it. These results are shown in table 6.

In an endeavor to study how the blood Ca and P are regulated at the time of parturition, the udders of two young goats about 8 months old were removed. It was considered that analysis of blood samples of these goats near the time of parturition would show whether the blood is fortified in these two elements in order to compensate for the demand on them for milk fever. Porcher (23) had used this method in the study of sugar metabolism connected with milk secretion. Only one goat became pregnant and at the age of about 14 months she gave birth to one kid which was found dead. The blood analyses for this goat are shown in table 7. It will be seen that there was a decided increase in blood P after parturition, and that this high level of blood P was sustained for some time. The Ca level of the blood remained quite constant throughout.

DISCUSSION

The data on blood sugar show that the sugar was generally lower after normal calving than before. This is brought out in table 1. The difference between the averages before calving and after is not large, but 9 out of the 13 cases showed lower blood sugar after calving than before. There seems to be a tendency for the sugar to rise at or near calving time and then to fall later, and this may explain some of the higher sugar values obtained very shortly before calving. It was found that when samples were taken within 10 hours after calving and then again over 10 hours after, the sugar in the second samples after calving was appreciably lower than in the first

TABLE 6

Ca and P = mg. per 100 cc. serum; Sugar = mg. per 100 cc. blood; Hb = per cent hemoglobin in whole blood; Al and Gl = per cent albumin and globulin in serum; phosphatase = phosphatase units

CASE NO.	TIME	BLOOD CONSTITUENT					
		Ca	P	Total sugar	Hb	Al	Gl
I Typical of milk fever	Before inflation	4.6	1.36	144.9			
	14 hrs. after inflation	9.3	4.11	101.9			
	48 " " "	9.0	8.99	56.8			
	7 days " "	9.2	4.86	34.6			
II Typical of milk fever	Immediately after inflation	4.9	.56	74.6			
	19 hrs. after inflation	9.3	6.37	55.6			
	43 " " "	9.8	10.53	41.2			
	6 days " "	9.2	6.61	48.8			
	14 " " "	9.5	6.02	30.3			
III Typical of milk fever	Before inflation	3.8	2.96	186.0			
	1 hr. after inflation	4.0	5.32	157.8			
	4½ hrs. after inflation	6.9	5.62	76.9			
	3 days after inflation	10.3	10.23	54.3			
	9 " " "	11.0	4.73	46.2			
IV Apparently on verge of milk fever. Udder not inflated	4 hrs. before calving	9.2	less than 1				
	5 " " "	7.1	less than 1	79.7			
	2 days " "	10.1	6.29	55.3			
V Typical of milk fever	15 hrs. before calving	10.6	4.96		12.8	3.77	2.26
	8½ hrs. after calving	6.0	1.52		13.6	4.15	2.40
	18 hrs. after calving, udder inflated	4.3	.88		13.5	3.96	2.12
	2½ hrs. after inflation	6.1	2.52		10.8	3.98	2.24
	1 day after inflation	7.8	5.82		10.8	3.94	2.14
	3 days " "	10.6	7.80			3.74	2.34
	5 " " "	10.8	8.28		12.6	3.75	2.02
	11 " " "	8.4	4.24		9.8	3.98	2.38
VI Typical of milk fever						Plasma Phosphatase	
	42 days before calving					.224	
	19 hrs. " "	10.0	3.86			.140	
	5 " after "	7.2	3.42			.175	
	29 " " "	5.1	2.44			.157	
	40 " " "						
	1 hr. after inflation	6.0	4.70				
	14 hrs. after inflation	7.7	6.40				
	2½ days after inflation	9.7	4.58				

samples, the averages being 91.2 mg. per 100 cc. for the first sample after calving and 59.9 mg. for the second. This is shown in table 2. Apparently some mechanism tends to increase the sugar of the blood at calving time in order to compensate for the drain on the blood for sugar used in the production of lactose, and to supply energy for milk secretion. This idea is in agreement with the results of Porcher (23) whose work has been men-

TABLE 7

Total Ca and inorganic P of the serum before and after parturition of a goat from which the udder had been removed

BEFORE PARTURITION

TIME	25 days	12 days	9 days	5 days	1 day
Ca mg. per 100 cc.	10.2	10.6	10.8	11.5	10.9
P mg. per 100 cc.	7.66	9.32	7.28	6.94	5.98

AFTER PARTURITION

TIME	1 day	3 days	11 days	20 days	
Ca mg. per 100 cc.	10.3	11.5	10.9	11.3	
P mg. per 100 cc.	11.52	9.36	10.26	7.68	

tioned previously. In the milk fever cases for which blood sugar determinations were made a hyperglycemia was found. The results agree with those of Fish (5) and others. Apparently high blood sugar is a result of milk fever and not one of the causes of it.

The results given in table 3 show that the blood proteins may vary considerably—particularly the albumin and globulin. However, the variations do not occur in any very consistent manner. In 4 cases out of 8, Nos. 1, 2, 6, and 8, the albumin was higher near the time of calving than before or after this time. In 4 cases out of 7, Nos. 2, 5, 6, and 9, the hemoglobin was higher near the time of calving than before or after this time. In the other cases the changes in albumin or hemoglobin do not appear to be of any significance. The rise of hemoglobin and albumin that did occur at calving time in the above cases might be taken as evidence of a slight amount of anhydremia. The globulin did not show the same tendency to rise at calving as did the albumin and hemoglobin. Two cases out of the 8, Nos. 2 and 4 showed a higher globulin near calving than before or after, and two, Nos. 1 and 6 showed a lower globulin than before or after. In the other cases the changes in globulin are not considered significant. If globulin does go over from the blood into the colostrum milk as suggested by the work of Howe (15), the rate at which it goes over is apparently not rapid enough to affect the globulin level of the blood to any great extent.

Milk fever case No. 4 on which blood proteins were determined does not indicate any appreciable amount of anhydremia. Hemoglobin was a little higher 10 hours before the attack of milk fever and also at the time the cow was down with the disease than it was before or after these times, but the differences are not very great. It is true that there was some fall in hemoglobin after the udder was inflated and the cow had recovered, but the albumin and globulin were quite constant throughout. This case clearly indicates that anhydremia is not an important factor in milk fever, and since the anhydremia theory is based essentially on indirect evidence it was not considered worth while to investigate it any further.

As stated previously the work done in the first part of this investigation did not show any consistent differences in the amounts of blood Ca or P before and after calving. However, the results of later work, in which more numerous samples were taken for each case, do bring out some interesting variations. These data, given in tables 3 and 4, show that there is a definite tendency for the Ca to fall shortly after calving particularly in cows which have been through one or more lactation periods. Every one of the ten cows which were not in their first lactation showed some fall in blood Ca some time during what is called the milk fever period. Of the 9 cows starting their first lactation only 5, Nos. 4, 6, 8, 21 and 25, showed such a fall in Ca. This is interesting when it is considered that milk fever rarely occurs at the first calving. In table 8 it is shown that the average Ca value for all cows reported is lower within one day of calving than it is before this period. The differences between these averages is found to be a statistically significant one when the data are analyzed by Student's method. The average value for Ca after the milk fever period, or over three days afer calving, is slightly lower than the average before calving, but the difference is not a significant one.

TABLE 8

Average Ca and P values of normal cows' serum at different periods around the time of calving

	Total Ca Mg. per 100 cc.	Inorganic P Mg. per 100 cc.
Over 1 day before calving	10.560	5.772
Within one day of time of calving	9.855	5.373
Over three days after calving	10.390	5.521

The blood P likewise shows a tendency to fall at the milk fever period, but this tendency is not so constant for P as it is for Ca. Twelve cases out of 19 showed a fall in P, but only 6 out of the 10 cows not in their first lactation showed this effect. In general P values vary much more than the Ca values, the range obtained for apparently normal cows being from 9.12 to 2.80 mg. per 100 cc. for P and from 12.1 to 8.3 mg. per 100 cc. for Ca. In some cases there was quite a marked rise in P around the time of calving. Table 8 shows that the average P values for all cows reported is highest in the period over one day before calving and lowest at the period within one day of calving, but the differences between these averages cannot be considered significant.

The blood Ca and P values obtained on all five milk fever cases, vary in essentially the same way as those reported by Fish (5). The Ca and P are both low at the time of milk fever and both rise as a result of inflation.

The Ca gradually returns to normal, recovery taking place when the Ca value reaches 5 or 6 mg. per 100 cc. The P rises quite rapidly after inflation and reaches values decidedly above normal—later returning to the normal level. A hypercalcemia following udder inflation such as Dryerre and Greig (3) have reported was not detected in any of these 5 milk fever cases. This condition may have occurred in some of these cases, but at least it was not present at the time the samples obtained were taken.

Case No. 4 in which the cow was apparently on the verge of having milk fever but did not come down with it is quite interesting. The P in this case was very low—estimated at less than 1 mg. per 100 cc., both before and after calving. After calving the Ca dropped to 7.1 mg. per 100 cc. It seems probable that what milk fever symptoms were present in this case, were due to the low Ca, and that low P has little to do with causing the milk fever symptoms. Palmer, Cunningham and Eckles (21) report one case of normal calving where the P dropped to 1.67 mg. per 100 cc., 5 days after calving.

TABLE 9

Plasma phosphatase values obtained for normal cows at different periods before and after calving

Phosphatase values = units of phosphatase as defined by Kay

COW NO.	LACTATION	TIME BEFORE CALVING				TIME AFTER CALVING		
		55–21 days	20–11 days	10–5 days	4–0 days	0–4 days	5–10 days	11–22 days
11	4th				.085	.095	.084	
12	3rd	.087			.195	.200	.179	.106
13	1st				.100	.106	.101	.200
14	4th		.070		.163		.145	.090
15	2nd			.086		.238	.178	
16	2nd		.168	.110		.180	.097	
18	2nd	.225			.114	.097	.085	
19		.182				.128		
20	2nd	.167		.103	.106	.193		.073
21	1st	.189	.150			.168		.074
22	8th	.162		.059		.094	.099	
24	3rd	.214		.089		.228	.154	
25	1st	.170	.110			.172	.094	
Ave.		.1745	.1265	.0894	.1271	.1583	.1247	.1062

The results obtained on normal cows before and after calving do not give much indication as to how the levels of Ca and P in the blood are regulated around calving time. The data obtained on the goat, which are given in table 7, suggest that there may be some mechanism which fortifies the blood in P in anticipation of the demand for it for lactation, but the blood Ca is apparently not influenced in this way. Greig (11) reports some data which indicate that whenever the blood Ca goes appreciably above or below normal, a reaction sets in which causes the Ca value to go in the opposite direction. No doubt the parathyroid glands would play an important rôle in such reactions. Gerschmann (8) has advanced the idea that there is a reciprocal relationship between the activity of the parathyroids and the blood Ca level. According to this idea when the blood Ca is low the parathyroids are stimulated to greater activity which tends to bring the Ca level up, and conversely, a high Ca level depresses parathyroid activity and this in turn tends to bring the Ca down. When we consider that the blood Ca is normally kept at a very constant level, it seems evident that it is controlled by some quite sensitive mechanism. Milk fever is essentially due to failure of the cow to maintain a sufficient amount of Ca in her blood. The abnormal drop in blood Ca, in milk fever, may be considered to result from one or both of the two following factors: first, an unusually large demand on the blood for Ca which the Ca regulating mechanism, though properly functioning, is unable to cope with; second, a failure of the Ca regulating mechanism to take care of the demand for Ca, which normally occurs at the onset of lactation. The first factor is probably the most important one.

The P value of the blood is quite markedly affected by various factors. This is brought out by the work of Palmer, Cunningham, and Eckles (21). Wide fluctuations in blood P can occur temporarily without causing any noticeable disturbances in the health of the animal. The low P values occurring in milk fever are probably the results of the conditions causing milk fever, and, apparently, low P has little to do with the etiology of the disease.

The data on plasma phosphatase before and after calving show considerable variation, but many cases vary in the same general way. There is a tendency for the phosphatase to be quite high, three weeks or more before calving, and then to gradually decrease till about the time of calving, when it goes up again. After calving it tends to gradually decrease again. However it is impossible to make any reliable interpretation of such data as our knowledge of phosphatase activity in the different tissues is quite limited. Interpretation is still more difficult on account of the fact that phosphatase in the plasma may be present as the result of a leakage or diffusion of this enzyme from various tissues of the body.

SUMMARY AND CONCLUSIONS

1. The blood protein values obtained in milk fever compared with those for normal calvings indicate that anhydremia is not an important factor in milk fever.

2. There is a tendency for the inorganic Ca of the blood of most dairy cows to fall slightly sometime within the first three days after calving. This tendency is most marked in cows that have been through a previous lactation period.

3. The inorganic P of the blood of dairy cows also tends to fall within the first three days after calving in many cases, but this tendency is not so consistent for P as it is for Ca. Blood P varies much more under the same conditions than blood Ca.

4. Additional evidence has been obtained that the essential factor in milk fever is a blood Ca deficiency.

5. Plasma phosphatase in dairy cows varies considerably. It tends to be fairly high three weeks or more before calving, then to fall till near the time of calving when it tends to go up. During the three weeks after calving it usually falls again.

6. The phosphatase activity of the milk is usually higher than that of the plasma of the cow, and the activity in the milk is much greater in cows at the end of lactation than in heavily producing cows at the beginning of lactation.

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ADDENDA

Since this paper was submitted for publication a paper has been published by Petersen, W. E., Hewitt, E. A., Boyd, W. L., and Brown, W. R., 1931, Jour. Am. Vet. Med. Assoc., LXXIX, 217; in which they describe the symptoms of hypoglycemia and hypocalcemia artificially induced in cows by the injection of insulin and sodium citrate solution respectively.

THE FAT PERCENTAGE OF MILK AS AFFECTED BY FEEDING MILK TO DAIRY COWS[1]

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INTRODUCTION

While the belief that feeds exert no specific influence upon the fat content of milk over long periods of time is generally accepted, the information in regard to the immediate effect of various feeds is somewhat conflicting. There seems to be reason for believing that certain feeds have a specific influence, for a short time at least, upon the fat percentage of the milk of cows to which they are fed. Such influence, if only of a temporary nature, is of little importance in its relationship to profitable production of butterfat. It is, however, a matter which is of considerable interest in its relation to the problem of milk secretion, and which may have some practical aspects in connection with the estimation of the yearly butterfat and milk production of individual cows under the systems of testing which are now in general use.

While many feeds have been studied in regard to their effect upon milk production and the composition of milk, no case has been found where dairy cows have been fed milk with the idea of determining its effect upon the composition of the milk produced.

REVIEW OF LITERATURE

The relation of feeds to butterfat percentage received much attention both in Europe and America during the last two decades of the nineteenth century and the first of the twentieth. Much of this work was not well controlled and in many cases involved small numbers of animals. While the results secured by different investigators often did not agree, there seems to be a general indication that certain feeds exert a specific influence upon the butterfat content of milk. This is particularly true of feeds of high fat content, and most notably of the oil bearing seeds and their products. The earlier American investigators rather consistently found the influence of feed to be of short duration and concluded that the effect was due to the change of feed rather than to any nutritional difference in the ration. This earlier work has been reviewed by Anderson (1) and by Clothier (5).

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Later investigators have uniformly found that feeds of high oil content such as flaxseed, peanuts, soy-beans, cottonseed meal, cocoanut meal and cocoa meal cause at least a temporary increase in fat percentage of milk.

Only one case was found where the effect of feeding milk was studied. Beger (3) using a sheep and a goat, found some indication that milk fat in the ration has a greater influence than other fats upon the fat percentage of milk. The following year Beger (4) made a more extensive study using three goats. His principal purpose was to study the relative effect of emulsified and non-emulsified fats in the feed upon milk production. He fed whole milk as a typical emulsified fat and skimmilk plus butterfat as a typical non-emulsified fat. Mixed feed with butterfat added was also fed to two of the animals, and in the case of the third, a period of mixed feed without butterfat and a period of skimmilk feeding were included. The two animals which had no periods without butterfat in the ration showed no variations between the different periods which could safely be attributed to the effect of the feed. The average fat percentage by periods of the third goat were as follows: whole milk, 3.75; skimmilk plus butterfat, 2.80; skimmilk, 2.45; mixed feed, 2.40; and whole milk, 3.25. The periods were of 10 to 12 days' duration with intermediate periods ranging from two weeks to two months in length. These results suggest that whole milk when given as a feed increased decidedly the fat content of this goat's milk while butterfat when fed with skimmilk had a similar effect but to a lesser degree. Skimmilk when fed alone apparently had no effect upon the fat percentage of the milk. Definite conclusions to this effect would not be justified since only one animal was involved.

EXPERIMENTAL

In a preliminary experiment, three Holstein-Friesian cows in the University herd, each producing about 40 pounds of milk daily, were fed whole milk with a fat content of about four per cent in amounts up to 36 pounds per day, in addition to their regular ration. None of the cows would drink liquid milk so it was necessary to add the milk to the dry grain ration. Some of the cows refused to eat the feed which was moistened with milk until a small amount of molasses was added to overcome the milk flavor. All attempts to teach cows to drink liquid milk were unsuccessful except in the case of those which were offered their own colostrum immediately after calving. Three cows were successfully taught to drink milk in this manner, although several refused. Of these three cows the one which apparently consumed the milk most readily suddenly refused to take the milk after it had been fed for nearly two weeks. Another of the three was removed from the herd because of disease. The third was fed thirty pounds of her own milk daily during alternate intervals and samples were tested from each milking. On the basis of observations made during these preliminary trials, an experiment was planned using eleven cows of three breeds and in various stages of lactation. Due to the difficulty of getting cows to drink milk, and since it apparently has essentially the same effect when given in the feed as when given in liquid form, the milk was fed by mixing with the grain ration in the proportion of two pounds of milk for each pound of grain. During the control periods an equal amount of water was substituted for the milk. Following a six day preliminary period, the cows were divided into two groups. One group was fed the regular ration moistened with whole milk and molasses while the other was fed the regular ration moistened with water and molasses. The experiment was continued through three six day periods in this manner, the groups being reversed at the end of each period. The whole milk used was a mixture of Guernsey and Jersey milk with an average test of 5.4 per cent. Since we were primarily concerned with the immediate effect upon the fat content of the milk and the total fat yield, no attempt was made to equalize the feed intake to allow for differences in the plane of nutrition due to addition of milk or cream. During the fifth period, part of the cows from each group were given the regular ration moistened with skimmilk and molasses, while the remaining cows of each group were given the regular ration moistened with cream containing 40 per cent fat, diluted with water and molasses. One pound of cream was fed for each three pounds of dry grain mixture, the water being added in order that the liquid content of the feed might remain the same during the different periods. These groups were reversed during the sixth period.

TABLE 1

Breed and Stage of Lactation of Cows Used in Experiment and Amount of Whole Milk, Cream, and Skimmilk Fed Daily

COW NO.	BREED	MONTH OF LACTATION	MILK DAILY DURING WHOLE MILK PERIODS	SKIM DAILY DURING SKIM-MILK PERIOD	CREAM DAILY DURING CREAM PERIOD
			pounds	*pounds*	*pounds*
155	Jersey	Ninth	28	28	4⅔
160	Jersey	Fourteenth	20	20	3⅓
178	Jersey	Thirteenth	20	20	3⅓
382	Holstein	Third	40	40	6⅔
387	Holstein	Third	40	40	6⅔
388	Holstein	Fifth	40	40	5
403	Holstein	Fourth	40	40	6⅔
412	Holstein	Thirteenth	28	28	4⅔
542	Guernsey	Fifth	24	24	4
544	Guernsey	First	24	24	4
545	Guernsey	Fourth	28	28	4⅔

In addition to the grain, all of the cows were given all of the alfalfa hay and corn silage that they would clean up readily. The grain mixture consisted of 250 pounds each of corn, barley, and oats; 100 pounds each of bran and linseed meal; and 50 pounds of cottonseed meal. Dried beet pulp was added at the rate of one pound for each four pounds of grain to increase the absorptive powers of the grain mixture and to lighten the wet feed. The average daily fat percentage of the milk of each cow was found by applying the Babcock method to samples prepared by taking an aliquot part from both the morning and the evening milk.

Table 1 shows the breed and the stage of lactation of each cow and the amount of milk, skimmilk, and cream received daily during the respective periods in which each was fed.

OBSERVATIONS

A six day preliminary period was used for all the cows included in this experiment in order that they might become accustomed to the change from dry feed to wet feed, and to the addition of molasses and beet pulp to their ration. The change apparently had little effect upon the fat percentage of the milk, as the tests were very uniform during this period.

The first feed of milk was given to the group receiving milk at the last milking of the preliminary period. The change of feed was made thereafter for each group with the last milking of each period, since the earliest possible effect of this feed would be upon the morning milking of the following day. Upon the first day following the change little effect upon the fat percentage was noted. Upon the second day, a marked increase in fat percentage was observed for the cows receiving milk. Some of the cows showed a slight effect on the first day after the change. The preliminary trials in which each milking was tested separately indicated that no effect was usually observed previous to the milking occurring twenty four hours after the change. This higher test was maintained during the entire six day period. No similar increase was noted in the control group. The groups were reversed at the end of six days. Upon the second day following the reversal, the cows which had been on the milk feed and were changed to the control diet, dropped in test, while those which had been on the control diet and were changed to the milk diet showed a decided increase. The groups were again reversed with similar results. This lag or latent period following the addition of milk or cream and the residual effect when they were discontinued confirms the observations of Nevens, Alleman and Peck (8) and of Aplin and Ellenberger (2).

During the fifth and sixth periods, in order to find which part of the whole milk was responsible for this increase in fat percentage, skimmilk and cream were used in the manner previously indicated. The cream apparently affected the test in practically the same manner as the whole

TABLE 2

The Effect Upon Milk Production of Feeding Whole Milk, Cream, and Skimmilk

Average daily milk production by periods

COW NO.	PRELIMINARY	NO MILK	WHOLE MILK	CREAM	SKIMMILK
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Group I					
155	29.23	29.89	29.17	26.82	29.65
160	18.32	19.45	19.70	19.55	19.50
382	53.33	55.65	53.70	56.40	55.52
387	59.82	59.04	57.98	54.15	58.28
403	57.27	58.04	56.98	58.42	55.73
545	29.62	33.44	31.57	30.57	32.57
Average of group	*41.26*	*42.59*	*41.52*	*40.98*	*41.88*
Per cent above or below control	*–3.12*	*0.00*	*–2.51*	*–3.78*	*–1.67*
Group II					
178	15.15	15.70	16.35	15.35	16.17
388	50.83	51.72	48.83	42.25	44.27
412	34.07	32.85	35.13	31.30	34.48
542	22.03	23.33	25.10	21.47	23.85
544	28.30	27.78	28.06	26.38	26.03
Average of group	*30.08*	*30.28*	*30.70*	*27.35*	*28.96*
Per cent above or below control	*–0.66*	*0.00*	*+1.39*	*–9.68*	*–4.36*

TABLE 3

The Effect Upon Butterfat Production of Feeding Whole Milk, Cream, and Skimmilk

Average daily butterfat production by periods

COW NO.	PRELIMINARY	NO MILK	WHOLE MILK	CREAM	SKIMMILK
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Group I					
155	1.52	1.55	1.91	1.61	1.58
160	1.13	1.20	1.40	1.38	1.12
382	1 93	1.93	2.22	2.11	1.78
387	1.74	1.73	1.98	1.87	1.73
403	1.92	1.82	2.11	2.16	1.62
545	1.43	1.57	1.78	1.72	1.47
Average of group	*1.61*	*1.63*	*1.90*	*1.81*	*1.55*
Per cent above or below control	*–1.23*	*0.00*	*+16.56*	*+11.04*	*–4.91*
Group II					
178	1.02	1.02	1.23	1.13	1.00
388	1.67	1.75	1.76	1.56	1.47
412	1.50	1.41	1.70	1.44	1.44
542	1.06	1.07	1.30	1.10	1.08
544	1.53	1.42	1.62	1.45	1.22
Average of group	*1.36*	*1.33*	*1.52*	*1.34*	*1.24*
Per cent above, or below control . . .	*+2.26*	*0.00*	*+14.29*	*+0.75*	*–6.77*

milk, while the skimmilk periods gave in most cases the lowest tests of the entire trial.

Table 2 shows the average daily milk yield, table 3 the average daily butterfat production, and table 4 the average butterfat percentage by periods for each cow. Since there seems to be a very definite tendency for the effect of each period to carry over until the second day following the change, it is thought that the true effect of the feed can best be shown by allowing for this lag in the effect. For this reason the averages given in these tables represent the period beginning with the second day following the change of feed, and including the first day following the succeeding change.

TABLE 4

The Effect Upon Butterfat Percentage of Feeding Whole Milk, Cream, and Skimmilk

Average butterfat percentage by periods

COW NO.	PRELIMINARY	NO MILK	WHOLE MILK	CREAM	SKIMMILK
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Group I					
155	5.22	5.17	6.54	6.01	5.32
160	6.19	6.16	7.11	7.07	5.73
382	3.62	3.48	4.14	3.74	3.20
387	2.92	2.93	3.42	3.46	2.96
403	3.35	3.13	3.71	3.70	2.90
545	4.84	4.69	5.63	5.63	4.51
Mean of group	*4.36*	*4.26*	*5.09*	*4.94*	*4.10*
Per cent above or below control	*+2.35*	*0.00*	*+19.48*	*+15.96*	*-3.76*
Group II					
178	6.71	6.50	7.49	7.36	6.19
388	3.29	3.38	3.60	3.70	3.32
412	4.41	4.28	4.83	4.60	4.17
542	4.81	4.58	5.19	5.12	4.53
544	5.39	5.11	5.78	5.48	4.68
Mean of group	*4.92*	*4.77*	*5.38*	*5.25*	*4.58*
Per cent above or below control	*+3.14*	*0.00*	*+12.79*	*+10.06*	*-3.98*

Chart I is a graph of the arithmetic mean of the daily fat percentages of each group during the whole milk and the control periods. Chart II is a similar graph for the cream and the skimmilk periods. The cows were grouped differently during the skimmilk and cream feeding periods because a part of the cows from each of the previous groups were changed to the skimmilk ration and a part were changed to the cream ration in order to show more clearly the constituent of the whole milk which was responsible for its effect.

No unfavorable physical effect which might be attributed to milk or cream feeding was observed even with the abrupt changes when these feeds

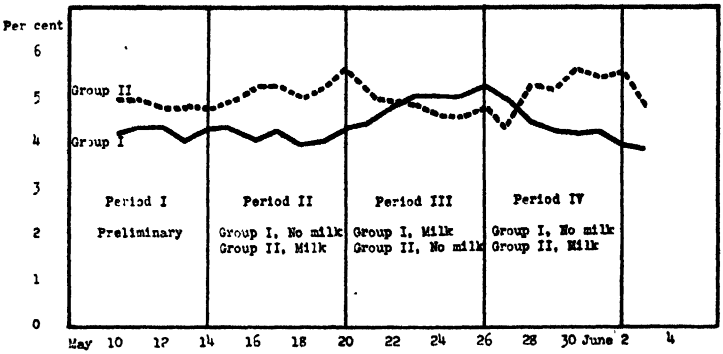

Chart I

BUTTERFAT PERCENTAGE AS AFFECTED BY FEEDING WHOLE MILK
(Group I, Cows 155, 160,382,387,403,545. Group II, Cows 178,388,412,542,544)

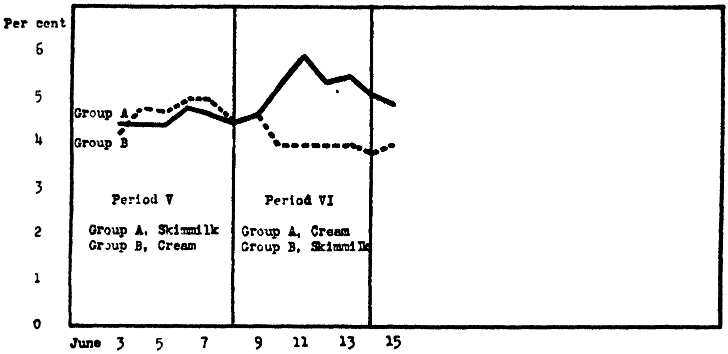

Chart II

BUTTERFAT PERCENTAGE AS AFFECTED BY FEEDING CREAM AND SKIMMILK
(Group A, Cows 155,178,387,412,542,545. Group B, Cows 160,382,388,403,544)

were added. Two of the cows showed a slight diarrhea at a time when they were on the control ration while one similar case was noted in a cow receiving whole milk.

The objectionable taste of the milk is apparently associated with the butterfat, since none of the cows objected to the feed mixed with skimmilk. Cows number 542 and 544 were especially obstinate in their dislike for whole milk and cream. While they usually cleaned up all of their feed before the next milking, they were very slow in eating the feed mixed with whole milk or cream. In nosing around over the wet feed, they squeezed out a part of the liquid, and probably actually consumed less cream or milk on some days than is credited to them.

DISCUSSION OF RESULTS

The uniformity of these results would seem to show conclusively that feeding of whole milk or of cream to cows has a very decided tendency to increase the fat content of their milk during the period when fed. This effect is brought about by an actual increase in fat production. While feeding of cream showed slightly less effect than whole milk upon fat yield and fat percentage, the individual results were not entirely uniform and it can not be stated definitely that this difference is characteristic of the two feeds. There seems to be little immediate effect upon milk production when whole milk is added to the ration of cows already being fed according to their requirements. With one group of cows the average milk production per period was greater during the period when milk was being fed, while in the other it was less. The milk production was lower during the cream feeding period for both groups as was indicated by the group averages, but the individual animals did not respond uniformly in this respect, as some of the cows had their highest milk production during the cream feeding period. The milk production during the skimmilk periods showed only slight variation from the control periods in which no milk was fed. The fact that cow number 388 was off feed during a part of the fourth and fifth periods and declined in milk production, has a tendency to decrease the average production of both milk and butterfat for Group II during the last three periods.

While the feeding of either whole milk or cream appears to bring about a marked increase in butterfat production and in butterfat percentage, skimmilk feeding gives no indication of increasing either of these factors. This indicates that the butterfat of the milk or cream or some constituent associated with the fat is responsible for the increased fat production and fat percentage.

The increase in fat production seems to be secured regardless of breed, stage of lactation, level of production, or condition of the cow. While the cows yielding milk with higher butterfat content showed a greater actual increase in test; if we consider this on the basis of the percentage of increase, there is not a large difference in the effect upon low testing and high testing cows.

The length of time for which milk feeding will continue to bring about increased fat production has not been determined. The cows which had a period of cream feeding immediately following a milk feeding period continued to produce a greater amount of fat than normal and to show a higher test for twelve days. In preliminary trials previous to this experiment, three Holstein cows showed a morked decrease in test of milk when milk feeding was discontinued after 16 days of feeding. Cow 388 of this experiment had previously been given 30 pounds of her own milk daily over two-week periods, and showed a decline in fat percentage each time the milk was discontinued, with a corresponding increase when milk feeding was resumed. Even though this effect may not be permanent, it might become a factor of practical importance in testing for butterfat production.

It is clear that the results of milk feeding can not be attributed to the change in plane of nutrition since the effects are not in line with those which we might expect from this factor alone. Eckles (6) has shown that when the plane of nutrition is lowered with cows in high condition, an increase in fat content of the milk usually results. Most of the cows of this group were in good condition, while some were in a high condition of fleshing. The lowering of the plane of nutrition when milk feeding was discontinued would be expected on the basis of the observations of Eckles to have a tendency to bring about an increase in fat content of the milk produced, which is entirely contrary to the results secured. Eckles and Palmer (7) found no significant effect on fat content of cows' milk due to an increase in the plane of nutrition.

The fact that the fat production and fat percentage merely returned to normal during the control periods and were not depressed to a subnormal level suggests that the effect of feeding butterfat in the form of whole milk or cream is probably a nutritional rather than a stimulative one.

The feeding of whole milk or cream to cows is a very inefficient method of increasing butterfat production as the increase in butterfat secured accounted for less than twenty per cent of the butterfat consumed by the cows.

SUMMARY

1. When whole milk was fed during alternate six-day periods to eleven cows in various stages of lactation, in addition to their regular ration, a marked increase in butterfat percentage and butterfat production resulted. This effect first became apparent about twenty-four hours after the milk was added and was maintained for an equal length of time after it was discontinued.
2. Similar results were obtained by feeding cream of high fat content.
3. The increased butterfat production secured by feeding whole milk or cream may be maintained for at least twelve days.
4. Skimmilk feeding did not bring about an increase in either butterfat production or butterfat percentage.
5. Definite conclusions in regard to the effect of milk feeding upon the milk production as shown by this experiment are not justified since the response of the different animals was not uniform. The results suggest that the immediate effect upon milk production is not significant.
6. The additional butterfat production due to feeding whole milk or cream was secured in a very inefficient manner, since the increase accounted for less than twenty per cent of the butterfat consumed.
7. Milk was not readily consumed by cows which were not accustomed to it, either as a drink or when mixed with their grain feed. Most cows, after becoming accustomed to the taste of milk, ate it readily when mixed

with feed. Some cows which had been taught to drink milk consumed the liquid milk readily.

8. No unfavorable physiological effect was observed in cows fed up to forty pounds of milk containing over two pounds of butterfat daily, even when added to the ration in the full amount after a period in which no milk was fed.

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CONTROL OF SPREADERS IN MILK PLATES[1]

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Our interest in the problem of the control of spreaders in milk plates was aroused by the occasional difficulties experienced in various milk laboratories. The problem was attacked from the point of the probable effect of differences in humidity in the air inside the agar plates, such as might occur with differences in the concentration of the agar used for the plates. A batch of 1.5 per cent agar was tubed and divided into five parts, which were subjected, respectively, to the various rates of drying which would occur in such places as a laboratory shelf, an incubator, an ice-box, a shelf over a radiator, and a closed tin receptacle, for a period of one week. When this agar was inoculated with spreaders and plated, the driest agar gave most definite evidence of the inhibition of the spreading tendency.

Thereupon, three different percentages of agar were prepared, with a still wider range in the relative agar-water content: 1.5, 2, and 3 per cent, according to the following proportions.

For every 1,000 cc. of tap water
3 grams Vegex.
5 grams Bacto-Peptone.
15, 20, and 30 grams of agar for the respective types of agar.

Each mixture was melted in an autoclave and filtered through a wet, hot, absorbent cotton filter; it was then tubed, exactly 10 cc. to a tube, and sterilized 30 minutes in the autoclave at 15 pounds pressure.

These three known concentrations of agar were then used in plating milk inoculated with one or more of a series of 12 different spreading organisms, obtained from recent routine milk plates in a large commercial plant. Raw, pasteurized, and sterile milk were used, also aged raw milk (4 days old); each lot of milk was inoculated with 1 cc. of a milk dilution of one or more spreaders, as indicated in Table 1.

The characteristic differences obtained with the three concentrations of agar are illustrated by the accompanying photographs; and the degree of spreading (number of spreaders and diffusion over the plate) is indicated in the table by * signs, ranging from *, indicating slight spreading, or 1–5 spreading colonies only to a plate, to *****, as in the 1½ per cent agar plates in figures 2 and 3.

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[1] Paper presented at the May meeting of the International Association of Milk Dealers in Washington, 1931.

TABLE 1

Degrees of spreading with total colony counts on 1½, 2 and 3% agar

INOCULATIONS	DILUTIONS	DEGREE OF SPREADING			COLONY COUNT		
		1½%	2%	3%	1½%	2%	3%
1. Agar, Spr. 1	10,000	*****	**	0	—	—	—
	100,000	***	***	0	28,600,000	28,080,000	30,000,000
2. " " 2	10,000	*****	**	0	—	—	—
	100,000	****	***	0	920,000	900,000	1,500,000
3. Milk (sterile), Spr. 4	1,000	**	**	*	—	16,000	24,000
4. " Spr. 5		**	**	*	64,000	68,000	67,000
5. " " 6		**	**	*	12,000	11,000	13,000
6. " (fresh, raw), Spr. 1	1,000	**	*	0	121,000	131,000	110,000
7. " Spr. 2	"	**	0	0	14,000	7,000	3,000
8. " " 3	"	**	0	0	26,000	10,000	14,000
9. " " 4	"	**	**	*	4,000	8,000	7,000
10. " " 5	"	***	**	*	34,000	32,000	25,000
11. " " 6	"	*	0	0	1,860,000	1,380,000	1,296,000
12. " " 4, 5, 6	"	*	0	0	1,662,000	1,860,000	1,800,000
13. " " 7	"	*	*	0	54,000	53,000	54,000
14. " " 8	"	**	*	0	53,000	74,000	45,000
15. " " 9	"	0	0	0	132,000	142,000	115,000
16. " " 10	"	0	0	0	220,000	210,000	198,000
17. " " 11	"	**	0	0	200,000	400,000	180,000
18. " " 12	"	**	*	*	75,000	110,000	68,000
19. " " 1	10,000	*	0	0	20,000	80,000	30,000
20. " " 2	"	0	*	0	600,000	530,000	540,000
21. " " 3	"	*	0	0	40,000	60,000	—
22. " " 1, 2, 3 and *coli*	"	*	0	0	2,940,000	2,880,000	3,360,000
23. " *coli* only	"	—	—	—	6,480,000	11,880,000	9,240,000
24. " (4 days old), Spr. 1	1,000	*	*	0	5,040,000	6,720,000	3,060,000
25. " Spr. 2	"	***	**	0	1,440,000	2,040,000	2,580,000
26. " " 3	"	*	0	0	7,080,000	3,000,000	4,020,000
27. " " *coli*	"	—	—	—	2,940,000	2,880,000	3,360,000
28. " " 1, 2, 3 and *coli*	"	**	*	0	3,840,000	2,880,000	2,440,000
29. " " 4	"	**	**	1	20,000	22,000	22,000
30. " " 5	"	**	*	0	87,000	170,000	210,000
31. " " 6	"	**	*	0	51,000	53,000	47,000
32. " " 4, 5, 6	"	*	*	0	30,000	85,000	107,000

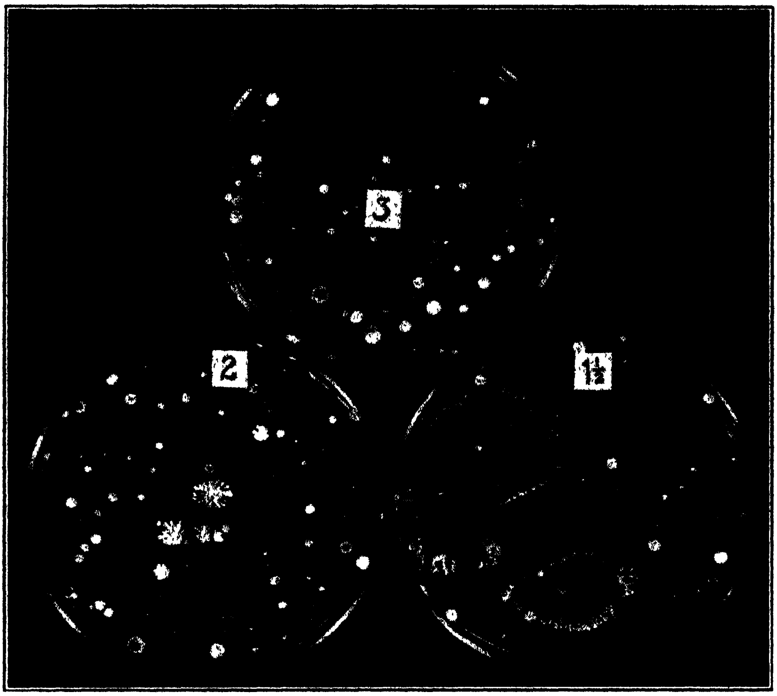

FIG. 1. TYPICAL RANGE IN BRANCHING, SPREADING COLONIES OBTAINED AFTER INCUBATION FOR 2 DAYS WITH 1½, 2, AND 3% AGAR

A high bacterial content was characteristic of most of the samples of milk used, and dilutions of 1,000 and 10,000 were usually used in making the plates; in a few cases 100,000 was also used. The weakest dilution is usually the one recorded in table 1. The more crowded of the high dilution plates were counted with the lantern-mirror device described by Broadhurst and Brown[2] which makes it possible to count crowded plates with a high degree of accuracy.

The total counts are, of course, important in this connection, since any medium which reduced the count per c.c. would give an erroneous report of the milk. The total colony counts are, therefore, given with the "spreader" reports in table 1.

To determine whether or not the higher concentrations of agar would limit the development of pin point colonies, which would militate against its use in milk examination, pin point organisms, obtained in a recent dairy laboratory outbreak, were used to inoculate milk (sterile and raw) rather heavily. As shown by the pin point results in table 2, pin point colonies develop about as readily in the 3 per cent agar as in the weaker concentrations.

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[2] Abstracts of Bacteriology, p. 10, January, 1925.

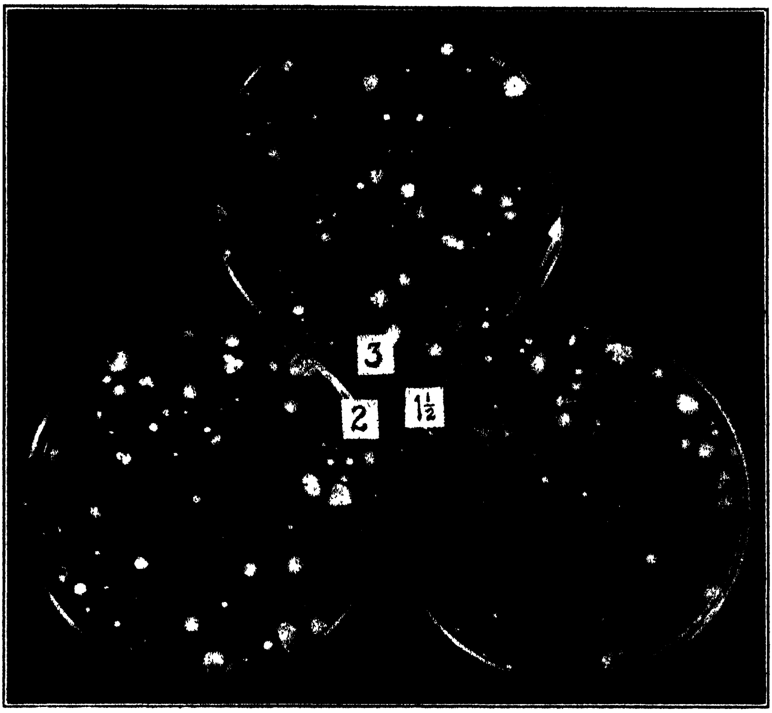

Fig. 2. Typical Range in Diffuse Type of Spreaders Obtained After Incubation for 2 Days With 1½, 2, and 3% Agar. (The Photography Was Delayed for Two Days, During Which the Slight Cracking Noticeable in 2 and 3 Occurred.)

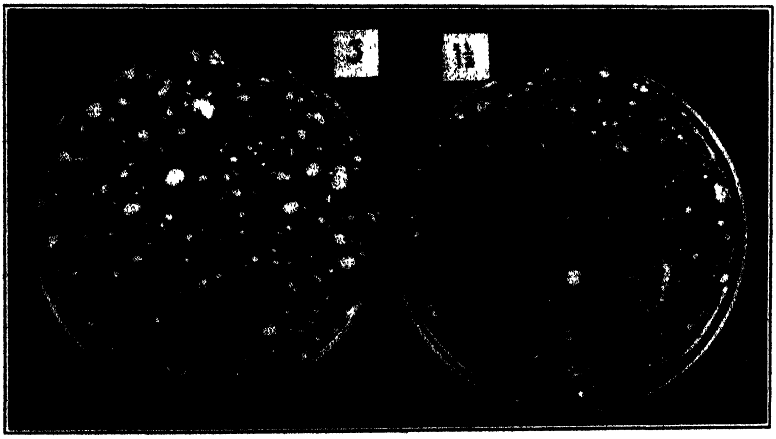

Fig. 3. Typical Range With Mixed Spreaders (Conditions as in Fig. 2)

TABLE 2

Pin point and total colony counts in 1½, 2 and 3% agar

INOCULATION OF PIN POINT CULTURE INTO	DILUTION	1½%		2%		3%	
		Pin point	Total	Pin point	Total	Pin point	Total
Milk (sterile) 1	1,000	32		78		17	
" 2	"	110		100		370	
" 3	"	51,300		25,820		——	
" 4	"	1,140,000		1,170,000		1,098,000	
Milk (raw) 5	100	172,000	179,700	90,900	98,000	95,400	106,600
" 6	"	245,700	260,400	234,000	249,300	253,800	278,000
" 7	"	385,200	395,000	419,400	435,600	312,200	357,800
" 8	"	198,000	235,200	181,000	219,600	179,400	247,800
" 9	"	108,600	114,000	270,600	277,700	300,000	329,600

Since the total count and the pin point count are not reduced by the use of 3 per cent agar, it can be recommended for routine milk examination as a medium which will limit the development of spreaders[3] without giving a false idea of the bacterial content of the milk.

If the higher 3 per cent agar is not used to replace entirely the 1.5 per cent agar in routine plating of milk, it could advantageously be used to make a duplicate plate for the weakest dilution, thus insuring a countable plate should spreaders give trouble on the ordinary agar.

CONCLUSIONS

1. The development of spreaders may be controlled by using higher concentrations of agar. The 2 per cent agar is not sufficiently helpful in reducing spreaders to recommend its adoption; but the 3 per cent agar causes a marked reduction in the spreaders.
2. The total bacterial count is not markedly or uniformly reduced by the 3 per cent agar.
3. The use of 3 per cent agar does not interfere with the development of pin point colonies.

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[3] Since the completion of this work (July, 1930, to April, 1931) the investigations of Boerner and Robinson on the Factors Influencing the Prevalence of Spreaders on Agar Plates in Bacteriologic Examination of Milk has appeared in the *Journal of Infectious Disease*, for April, 1931. The experiments described by these workers eliminated the various environmental factors in the laboratory as sources of spreaders, thus relating the presence of spreaders to the milk itself and the pre-laboratory conditions. Their results lead them to conclude that spreaders are inhibited by using limited amounts of agar in the plates—10 c.c., rather than 15 c.c. or 20 c.c. Spreading was also affected by the use of a dehydrating agent (calcium chloride) in the incubator, which changed the agar concentration from 1½ to 2½ per cent. There is some parallelism in their results and ours, as we find that spreaders are less in the more concentrated agar plates. Our work, however, was all done with carefully measured 10 c.c. amounts, and contrary to their reports, spreaders can develop luxuriantly on 10 c.c. plates, as illustrated by our photographs.

SOME FACTORS INFLUENCING THE CAPACITY OF THE ATMOSPHERIC DRUM DRIER

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Dry milk prepared by the atmospheric drum process in American plants was at one time considered suitable only for stock food. During recent years there has been a growing tendency to dry milk, principally skimmilk and sweet cream buttermilk by this process for human consumption.

Though dry milk made by the spray process is more completely redispersed into water than that made by the atmospheric drum process, little or no evidence is available which would indicate that this property of dry milk is of any great importance to the present users of the product. True, if dry milk is to be reconstituted and sold as fluid milk the product made by the spray process would need be used; however very little, if any, market milk is now being prepared from the dry product nor is likely to be prepared in the near future. It is likely, therefore, and quite logical that greater uses will be found in the future for drum process dry milks such as dried skimmilk and sweet cream buttermilk.

Much work has been conducted in studying the physical and chemical properties of dry milks. Considerable attention has been given by investigators to the keeping qualities, degree of dispersion in aqueous solution, manufacturing procedures effecting the dispersion, and to the cause and prevention of dry milk defects. By far the greatest amount of this work has been conducted with dry milks made by the spray process.

Little or no work is found in the literature on the operation of the drum drier. Information now available on the operation of such machines is to be found in publications issued by the manufacturers interested in the sale and distribution of drum driers. It was the object of the work here reported to study a few of the possible factors influencing the capacity of the drum drier. The work was prompted on interviewing a number of operators of the drum drier, from whom it was learned that little or no uniformity existed in the procedure of operation. It was observed that in some plants cold milk was passed on to the hot drums, while in others the milk was preheated to varying temperatures and passed on to the drums in a hot state. It was this fact, primarily, that induced the writers to consider the subject of the factors influencing the capacity of atmospheric drum driers.

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[1] Paper No. 1035, Journal Series, Minnesota Agricultural Experiment Station. The data here presented constitute a portion of those assembled in the thesis of E. H. Hubbard, submitted in partial fulfillment for the degree of Master of Science, University of Minnesota, June, 1930.

EXPERIMENTAL

A number of the possible factors influencing the capacity of an atmospheric double drum drier were considered. The following factors were included in the work here presented.

1. Temperature of milk when applied to the drums.
2. Amount of milk over drums—referred to as the "milk level" and indicated as "low," "high," and "normal."
3. Steam pressure and speed of drums.
4. Concentrated milk.

Skimmilk was dried on an atmospheric double drum drier manufactured by the Buffalo Foundry and Machine Company. The size of the drums was 24″ x 36″, rated with a capacity of 40 pounds per hour when skimmilk is dried and 90 pounds per hour when concentrated skimmilk of 25 per cent solids is dried.

Fresh skimmilk of a titratable acidity of not to exceed 0.2 per cent was pasteurized at 145° F. for 30 minutes and cooled to below 50° F., at which temperature the milk was held until desiccated. From 600 to 1000 pounds of skimmilk were used with each trial containing a representative solid content for the Twin City area of 9.2[2] per cent solids. Concentrated skimmilk containing 30 per cent milk solids was desiccated in determining the influence of this type of milk on capacity of the drier.

A standard method of drying milk on the drum drier was adopted as follows: Milk was preheated to a temperature of 150 to 160° F. and passed on to the hot drums. The milk was maintained at a level over the drums to a point just below the bottom of the splash plates and even with the top of the drums. A steam pressure of 60 pounds was used on the drums at a speed of 24 r. p. m. The capacity of the machine under these conditions was found to be about 58 to 60 pounds per hour. This capacity is well above the rated capacity of the machine, probably due to the slight increase in speed used over that recommended by the manufacturer.

The drums were put into motion after which the steam was turned on slightly and gradually increased until the steam gage recorded 60 pounds. The drums were then allowed to heat for 10 to 15 minutes after which the knives were adjusted and milk allowed to flow on the drums. After the machine had been in operation for some time the capacity study was conducted. Particular care was taken to make changes in operating conditions slowly. The capacity of the machine under a given condition was then determined by weighing the dry milk produced in a given time.

The moisture content of dry milks produced in a number of instances was determined using the method suggested by Bidwell and Sterling.[3]

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[2] Composition of skimmilk for the Twin City area will average 9.2 per cent total solids during the winter and 9 per cent during the spring and summer months.

[3] Bidwell, C. L., and Sterling, W. F. 1925. Preliminary notes on the direct determination of moisture. Indus. and Engin. Chem. 17: 147.

Solubility determinations were made on these samples using a modification of the method used by Supplee.[4] The method followed consisted in agitating 20 grams of the dry milk with 200 cc. of distilled water at room temperature for 15 minutes. A 50 cc. portion of the reconstituted milk was transferred to a centrifugal tube and centrifuged at 1000 r. p. m. for 15 minutes. A total solids determination was then made on a 2 gram sample of the supernatant liquid. The solubility was then expressed in percentage of moisture free dry milk dispersed in the supernatant liquid.

INFLUENCE OF TEMPERATURE OF MILK ON CAPACITY OF THE DRUM DRIER

Skimmilk of varying temperatures, ranging from a cold state (50 to 60° F.) to 185° F. was applied to the drums operated under standard conditions. The results are shown in table 1. Operation of the drier under standard conditions, which included applying the milk to the drier at 155° F., resulted in the production of 58 pounds of dry skimmilk per hour. Cold skimmilk applied to the drum resulted in a capacity of 53 pounds per hour, a reduction of 5 pounds, or 8 per cent. By heating skimmilk to 175° F. before applying to the drums, a production of 67 pounds of dry skimmilk per hour, or an increase of 15.5 per cent over the capacity of the drier when skimmilk was applied at 155° to 160° F., was obtained. A capacity of 69 pounds of dry skimmilk per hour was secured when the skimmilk was applied on the drums at 185° F., or an increase of 18 per cent when compared with the capacity secured under standard operation.

TABLE 1

The Influence of the Temperature of Skimmilk when applied to the Drums on the Capacity of the Atmospheric Drum Drier

TRIAL NO.	TEMPERATURE OF MILK WHEN APPLIED TO DRUMS	DRY SKIMMILK PRODUCED	TIME TO DESICCATE	CAPACITY PER HOUR
	°F.	pounds	minutes	pounds
1	50–60	40.00	45	53.0
2	50–60	15.00	17	53.0
3	125	9.25	10	55.5
4	155	32.00	33	58.0
5	160	18.5	19	58.0
6	175	39.0	35	67.0
7	185	15.0	13	69.0

The significant data here presented, were the striking differences in the capacity of the atmospheric drier when milk at a low temperature (50 to 60° F.) was applied to the drums as compared with the capacity of the machine when milk was heated to 175 to 185° F. previous to drying. There was an increase of 26 to 28 per cent due to applying the hot milk on the drums as compared with cold milk.

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[4] Supplee, G. C., and Bellis, B. 1925. Jour. Dairy Sci. 7: 39–46.

The Effect of the Milk Level over the Drums on the Capacity of the Drum Drier

The level of milk over the drums was varied to a point well below and above that used under standard operation. The influence of a reduced and increased milk level on the capacity of the drier is shown in table 2.

TABLE 2

The Influence of the Milk Level over the Drums of the Capacity of the Atmospheric Drum Drier

TRIAL NO.	MILK LEVEL	AMOUNT OF DRY MILK	TIME REQUIRED TO DESICCATE	CAPACITY PER HOUR
		pounds	minutes	
1	Low	17.25	21.0	49
	Normal	32.00	33.0	58
	High	16.25	13.5	72
2	Low	13.00	24.0	32
	Normal	13.50	17.0	47
	High	15.00	14.0	64

The results show conclusively that when the drier was operated with a low level of milk over the drums the capacity of the machines was greatly reduced. Likewise, an increase in capacity is secured by increasing the level of milk well above the drums. The low capacities reported on the batches of skimmilk dried in trial 2 are due to the difficulty experienced of establishing the normal level. The results of the two trials, however, are well in accord.

In comparing the capacity of the machine at different milk levels, it is observed that an increase of 40 pounds per hour, or 80 per cent, was secured when a high level was used in trial 1 as compared with the result secured in trial 2 when a low milk level was used on the drums. For maximum capacity, the milk should be held at as high a level as possible and yet avoid spillage.

Influence of High Steam Pressure and High Speed of Drums on Capacity of Drier

Some difficulty was experienced in attempting to vary the speed of the drums and the steam pressure at the same time when skimmilk was dried. When a high speed was attempted with a low steam pressure the resulting film became too wet or rolled at the knives. Since it was found that when skimmilk is dried a radical change in speed required a similar change in steam pressure it was deemed advisable to combine the two factors as one. The drier was operated at the standard speed and steam pressure and at a speed of 36 r. p. m. and with 85 pounds steam pressure on the drums. Milk was applied on the drums in a cold state, at 155° F. and 185° F. The influence of this procedure on the capacity of the drier is shown in table 3.

TABLE 3

Influence of Steam Pressure and Speed of Drums on the Capacity of the Drum Drier

TRIAL	TEMPERATURE OF MILK	STEAM PRESSURE	SPEED OF DRUMS	AMOUNT OF DRY MILK PRODUCED	TIME REQUIRED TO DESICCATE	CAPACITY PER HOUR
	°F.	pounds	r. p. m.	pounds	minutes	pounds
1	50 to 60	60	24	40.0	45	53.0
	50 to 60	85	36	16.0	15	64.0
2	155	60	24	32.0	33	58.0
	155	85	36	14.5	12	72.5
3	185	60	24	15.0	13	69.0
	185	85	36	17.0	12	85.0

It is evident from the data secured that regardless of the temperature at which milk is applied to the drums an increase in the steam pressure and speed of the machine resulted in an increased capacity. The maximum capacity was secured when skimmilk was preheated to 185° F. and the machine operated at a speed of 36 r. p. m. and a steam pressure of 85 pounds used on the drums. It is apparent that when efficient operation is desired, high milk temperatures should be accompanied with increased speed and temperature of drums.

The capacity of the machine when cold milk was applied to the drums and operated at 24 r. p. m. using 60 pounds of steam pressure in the drums was 53 pounds per hour. An increase of 32 pounds per hour, or 60 per cent, was secured by preheating the milk to 185° F. and operating the machine at 36 r. p. m. and using 85 pounds steam pressure in the drums.

THE INFLUENCE OF DRYING CONCENTRATED SKIMMILK ON THE CAPACITY OF THE ATMOSPHERIC DRIER

It is conceded that the most efficient means of removing the water of milk is by boiling milk under vacuum. Though it is known in a general way that the capacity of the drier can be increased by precondensing before drying on the atmospheric drum drier, no experimental data are available on the subject. Concentrated milk at 40° F. was applied to the drums of the drier.[5] The speed of the drums was 34 r. p. m. in the trial made using cold concentrated milk and from 20 to 36 r. p. m. with concentrated milk at 180° F. The data secured are shown in table 4.

The most striking result of this experiment was the capacity of the drier when concentrated milk was heated to 180° F. and passed on the drums operated at 36 r. p. m. (trial 5). In this instance, the capacity reached 130 pounds of dry skimmilk per hour. Of interest, also, was the capacity of the drier of 70.2 pounds per hour when cold (40° F.) concentrated milk (trial

[5] The drying of the milk in this trial was supervised by S. T. Coulter of the Division of Dairy Husbandry of the University of Minnesota.

TABLE 4

The Capacity of the Atmospheric Drum Drier as Influenced by Concentrated Milk

Concentrated milk applied to drums at 40° F. and 180° F. Steam pressure on drums 80 to 85 pounds. Speed of drums varying from 20 to 36 r. p. m. Concentrated milk containing 30 per cent total solids.

TRIAL NO.	TEMPERATURE OF MILK	SPEED OF DRUMS	DRY MILK PRODUCED	TIME REQUIRED TO DESICCATE	CAPACITY PER HOUR
	°F.	r. p. m.	pounds	minutes	pounds
1	40	34	21.0	18	70.2
2	180	20	50.0	41	73.2
3	180	28	82.0	49	100.0
4	180	34	10.5	6	105.0
5	180	36	74.0	34	130.0

1) was applied to the drums when operated at 34 r. p. m. and the capacity of 73.2 pounds per hour when concentrated milk at 180° F. was passed on the drums operated at a speed of 20 r. p. m. The influence of the speed of drums on the capacity of the drier was secured with less difficulty when concentrated milk was used by operating the drier with 80 to 85 pounds steam pressure than was experienced in the case of skimmilk. In trials 2, 3, 4, and 5, the speed of the drums was 20, 28, 34, and 36 r. p. m. respectively. The capacity of the drier was increased from 73.2 pounds of dry milk per hour when operated at a speed of 20 r. p. m. to 130 pounds per hour when operated at 36 r. p. m.

OTHER FACTORS INFLUENCING THE CAPACITY OF THE ATMOSPHERIC DRUM DRIER

The experience gained in the work here reported suggested to the authors a number of other factors which may possibly influence the capacity of such machines as the atmospheric drum wrier. Though no data are here presented, it is the belief of the writers that atmospheric conditions, particularly temperature and humidity, are important factors affecting the capacity of such machines as they are in the case of spray drying machines. The velocity of the air over the rolls doubtless affects the capacity of the drier as well as its utilization of steam. In the conduction of the work here reported no attempt was made to control the temperature, humidity, or velocity of air coming in contact with the drum drier. It is felt in some instances that the above conditions influenced data on some factors studied but not here reported and on which further work will need to be completed.

SOLUBILITY AND MOISTURE AS EFFECTED BY THE RATE OF DRYING MILK ON THE ATMOSPHERIC DRUM DRIER

The evidence presented above indicates quite clearly that there may be a wide variation in the rate at which dry milk is produced on the atmospheric drum drier. The question naturally arises, is the increased capacity accomplished at the expense of increased moisture in the finished dry milk and is the solubility of the dry milk affected? Since the moisture and solubility determinations were not made on all samples, the determinations presented in table 5 represent occasional samples from the trials conducted on the effect of milk level, influence of high steam pressure and high speed, and the influence of drying concentrated skimmilk on the capacity of the drier. It will be observed from the data presented that when the drier was operated under conditions which result in an increased capacity of the machine that there was no effect on the moisture content of the resulting dry milk. It was further observed that the solubility of the dry milk was in no way influenced due to varying rates or drying. This statement however does not include conditions of drying which may have resulted in an excessively dry film near the knife nor conditions which encouraged the production of burnt milk on the roll.

TABLE 5

Relation of Amount of Dry Milk Produced per hour on the Drum Drier to the Moisture Content and Solubility of Dry Skimmilk

SAMPLE	DRY MILK PRODUCED PER HOUR (CAPACITY)	MOISTURE	SOLUBILITY
	pounds	per cent	per cent
1	49	3.6	72
2	58	3.6	72
3	64	3.2	71
4	70	3.7	73
5	72	4.2	71
6	72	3.2	72
7	85	3.8	72
8	100	3.0	72
9	105	3.2	72
10	130	3.4	72

SUMMARY

Some of the possible factors responsible for variations in the capacity of atmospheric drum driers are suggested. A standardized method of procedure was set forth for the operation of the drier. The influence on the capacity of the drier on variations from this procedure are reported. Under standardized or normal operation it was stated that the capacity of the drier was from 58 to 60 pounds per hour. Drying of cold milk (50 to 60° F.) resulted in a decrease of 8 per cent in the capacity of the machine. By preheating skimmilk to 185° F., an increase in the capacity of the machine of 18 per cent was secured. It was noted that 26 to 28 per cent more dry skimmilk resulted per hour when skimmilk was applied to the drums at 180 to 185° F. than when skimmilk was applied on the machine in a cold (50 to 60° F.) state.

The level at which the skimmilk is maintained over the drums was shown to materially affect the capacity of the drier. In one instance the capacity of the machine was reduced to 32 pounds per hour when a low level was maintained, and in another, when a high level was used, there was a yield of 72 pounds of dry skimmilk per hour, a difference of 40 pounds per hour.

The influence on capacity of the steam pressure in the drums and the speed of the machine was studied. With an increase in speed of drums to 36 r. p. m. and an increase in steam pressure in the drums to 85 pounds, the capacity of the machine was 85 pounds of dry skimmilk per hour. Regardless of the temperature of the milk when applied to the drums, the increased speed and steam pressure resulted in increased capacity of the machine. The greatest capacity was secured, however, when the temperature of the milk was applied at the higher temperature (185° F.) using a high speed of machine and high (85 pounds) steam pressure.

The most marked influence on the capacity of the drier was noted when concentrated skimmilk containing 30 per cent solids was dried. When cold (40° F.) concentrated skimmilk was applied to the drums operated at 34 r. p. m. a capacity of 70.2 pounds of dry skimmilk per hour resulted. By reducing the speed of the machine to 20 r. p. m. and applying milk at 180° F. to the drums, a capacity of but 73.2 pounds of dry skimmilk resulted. By using the same steam pressure, increased speed of drums up to 36 r. p. m., and applying concentrated milk at 180° F., the capacity of the machine was increased to 130 pounds per hour, or 116 per cent increase over that secured under the standardized method adopted.

Though some variation was observed in the moisture content of dry milks produced on the drum drier, it was not possible to correlate variations in moisture of dry milk with the rate at which dry milk was produced from the dryer. With milk of uniform freshness it was evident that variance of the capacity of the drier had no effect on the amount of dispersion of dry milk when reconstituted with water.

CONCLUSIONS

The capacity of atmospheric drum driers is influenced by the following conditions:

1. Concentration of milk.
2. Temperature of milk.
3. Steam pressure used in drums.
4. Speed of drums.
5. Level at which milk is maintained over drums.

CHEESE SPREADS[1]

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The term cheese spread may be applied to any form of packaged cheese that is of such consistency that at ordinary room temperature it may easily be spread with a knife. There are several types of such products on the market:—(1) Cream cheese with pickles, olives, et cetera added, (2) processed cheeses of such age and moisture content as to be "spready," (3) processed cheese with concentrated whey or skim milk powder added and of such fat and moisture content that the mixture will spread easily. This article concerns itself with cheese spreads of the latter type.

Cheese spreads of this type may easily be distinguished from those of the second type by the presence of the sugar. In a moisture determination, especially if higher temperatures are used, as is commonly the practice with cheese, the sugar caramelizes, giving the mass a dark brown or black appearance. Moisture determinations on such products can therefore be made only with the use of low temperatures and preferably under vacuum.

As far as the authors know there are at this time no legal standards to govern the fat and moisture content of such cheese spread. Table 1 gives the composition of five typical commercial products of this type and of

TABLE 1

Analyses of cheese spreads

SAMPLE NO.	MOISTURE	FAT	FAT IN SOLIDS	SUGAR (LACTOSE)
	per cent	*per cent*	*per cent*	*per cent*
1	41.13	30.66	52.08	2.13
2	43.38	25.48	45.00	5.39
3	44.19	25.63	45.92	6.75
4	44.89	25.16	45.65	4.28
5	44.05	33.27	59.46	4.20*
6	39.62	28.28	46.83	6.25**
7	41.40	30.51	52.06	6.25**
8	38.29	31.57	51.16	6.25**
9	40.74	32.17	54.29	6.25**
10	40.45	33.78	56.72	6.25**
11	39.67	33.87	56.14	6.25**
12	37.80	33.65	54.10	9.50**

* Calculated from figures given by the manufacturer.

** Estimated from the amount of whey powder used.

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[1] Published with the permission of the Director of the Wisconsin Experiment Station.

seven experimental samples. These products are made by methods entirely similar to those used in the manufacture of processed cheese. The ground cheese plus such products as condensed whey, whey powder, or skim milk powder, is processed with the addition of sodium citrate, di-sodium phosphate or Rochelle Salt; water is added according to the mixture used and the consistency desired in the finished product. The desired consistency can also be obtained by using cream instead of water thereby utilizing both the increased fat and moisture content to produce the spreading quality. Usually a small amount of common salt (0.5 per cent or less) is added.

It will be noted that the mixture used in making these spreads, and the composition arrived at, depart appreciably from cheese. As a result they cannot be sold as cheese; actually they are sold as food products under proprietary trade names. Recently the authors made a study of the composition of cheese spreads to determine whether they could be made to conform more closely to the composition of cheese (with respect to fat and moisture content) without sacrificing the desired spreading quality. In harmony with this, the aim was to make spreads with a moisture content of about 40 per cent and with varying fat contents to determine the effect of the fat on the spreading quality. For this purpose mixtures of cheese (old, well-cured), whey powder and cream were processed. The amount of whey solids that may be used is limited by the solubility of the lactose as well as by flavor considerations. In the experimental work the amount of whey powder used was 10 per cent of the mixture. The solubility of lactose at 15°C. (59°F.) is 16.9 parts per 100 parts of water. On the basis of a 40 per cent moisture in the cheese spread, a lactose content of 6.76 per cent would constitute a saturated solution. Since the whey powder contains approximately 65 per cent lactose, 10 pounds of whey powder per 100 pounds of mixture would contribute 6.5 pounds of lactose. That this amount cannot be exceeded to any great extent is indicated by the last sample in table 1 where 15 per cent of whey powder was used and in which very extensive lactose crystallization occurred. The crystals shown in figure 1 were isolated from this sample.

The mixtures that were made consisted of 10 parts of whey powder and 87.5 parts of cheese, cream, and water, 2.0 parts of sodium citrate and 0.5 part common salt. The proportions of cheese, cream and water were varied to produce the desired fat content with a moisture of 40 per cent.

The calculations necessary to arrive at the required mixtures to attain a specified composition must be based upon the fat and moisture content of the cheese, the fat, solids-not-fat and moisture content of the cream and the moisture content of the whey powder. On account of the fact that some of these values were estimated and because of unavoidable evaporation losses in processing, the desired composition was not accurately obtained in all cases. Typical calculations are appended to this article because they are of interest and may find practical application.

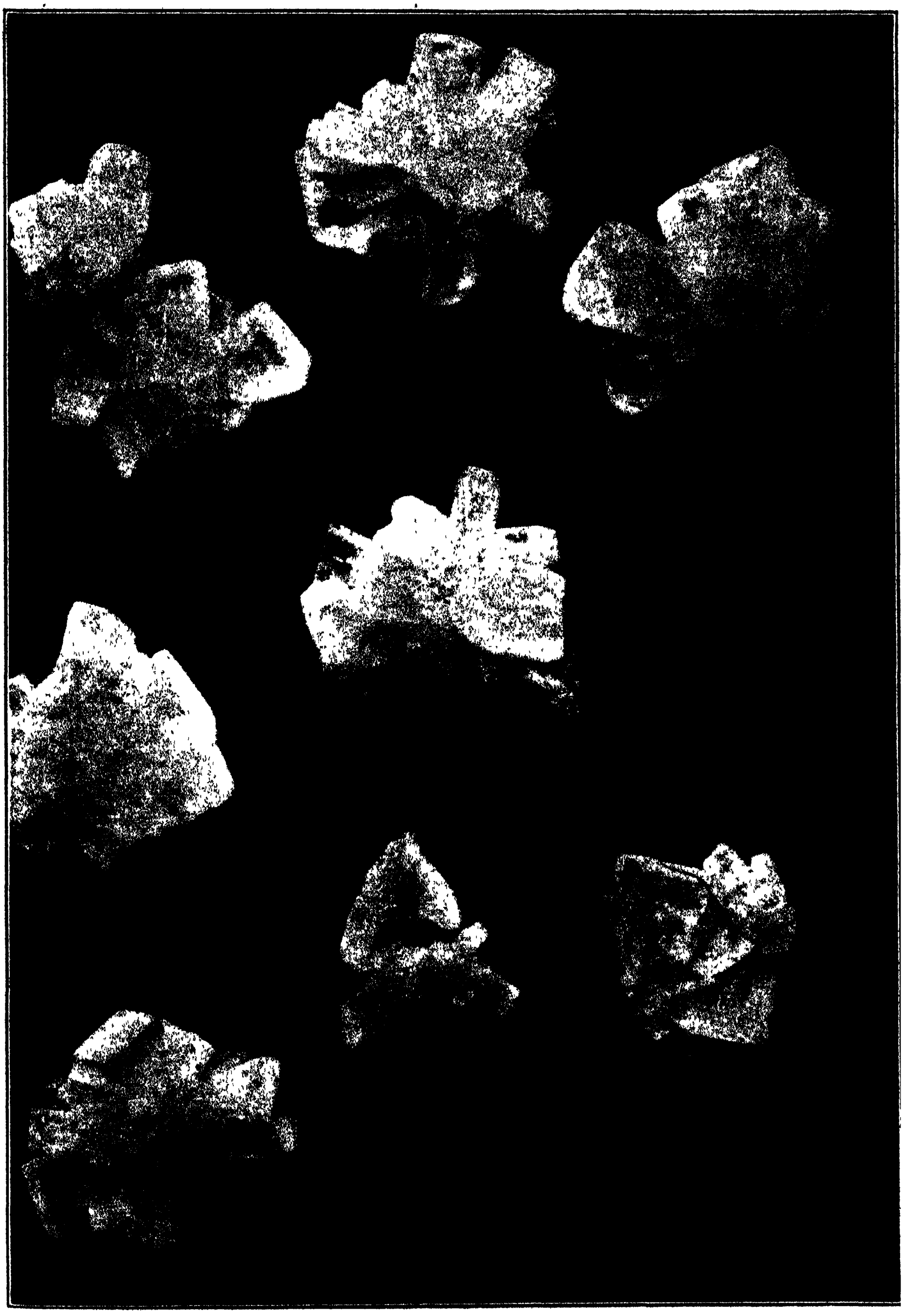

FIG. 1. LACTOSE CRYSTALS ISOLATED FROM A CHEESE SPREAD. MAGNIFICATION 8 ×

Having calculated the amounts of the constituents to be used, the next step in the process was the blending and heating. These operations were performed at the same time in an ordinary processed cheese kettle. Part of the cheese was put in the kettle and some of the water or cream added. The emulsifying agent to be used was added either in the solid form or dissolved in the water, when the solid was used there was no difficulty as sufficient water was present to dissolve the crystals almost immediately. The whey powder was then added slowly, and the remainder of the cheese, cream and water. The heating of the mixture was started any time after the whey powder had been added. Due to the rather moist nature of the product it was heated to a somewhat higher temperature than that used for processed cheese. Another reason for the higher temperature lies in the fact that the addition of pasteurized cream decreased the acidity of the mixture (increased the pH value) and increased the number of heat resistant, putrefactive organisms present, in addition to those in the cheese, and unless these organisms were killed at this time they would grow under the less acid conditions producing changes that would render the cheese very offensive and unfit for human consumption. For these reasons temperatures of 160° to 180°F. are to be recommended. If the lower temperature is used it should be maintained for at least ten minutes, water being added from time to time to keep the moisture content at the desired level. The amount of water to be added can be determined only by practice with the kettle used.

The effectiveness of the higher temperature treatment was increased by the method in which the spread was packaged. The hot mixture was filled directly into the tin foil lined wooden boxes and the top layer of foil turned over the cheese as soon as possible in order that it would cling more closely to the cheese. The boxes were made of $\frac{3}{8}$ inch lumber and retained the heat very well as they were still warm the next morning. That this slow cooling plays a distinct part in commercial work has been shown in numerous instances by the spoilage of the outside rows of containers in a large stack of boxes while those in the center, that were kept warm for a longer time, kept perfectly.

The sanitary measures used in handling the hot cheese were the same as those employed in the handling of any food product. If old cheese is used or if for other reasons the texture of the product tends to be coarse, it is possible to produce a smooth texture by running the hot cheese mass through a mustard mill, paint mill, or homogenizer. If this method is used, the additional step in the process may be a very fertile source of contamination. Care should be exercised to keep the equipment in a sanitary condition and it is advisable to reheat to at least 160°F. in case the temperature has been allowed to drop.

From the studies that have been made in this laboratory on experimental cheese spreads, samples that have been sent in for inspection and those purchased for study from local retail merchants, there are a number of rather definite conclusions that may be drawn. The spreading quality is due primarily to the fat and moisture content of the sample. In some samples having a rather excessive amount of moisture this factor seemed to be primarily responsible for the spreading quality, but in most cases a somewhat lower moisture content with a higher fat content gave a very satisfactory product. In samples having less than 40 per cent moisture with a corresponding increase in the amount of fat present the product seemed to spread as well as those in which the amount of water exceeded 40 per cent. When the spreading quality was due primarily to the high moisture content, the texture of the sample may be best described as mealy, but as the fat content increased this mealiness disappeared and the texture became more like that of butter. This was shown very clearly in samples 6 to 11 inclusive in table 1. Numbers 10 and 11, having an especially high fat content, were very soft and resembled soft butter. The others were much better in that they did not flatten out as rapidly when removed from the containers. All of these samples spread very easily and smoothly; number 6 showed a slight tendency to be mealy but this was not objectionable. When fresh, sample number 12 had a very desirable spreading quality and this was still evident even after the formation of lactose crystals made it impossible to spread the product in a smooth, buttery manner.

The reaction of cheese spreads varies between rather wide limits depending upon the composition. If there was little or no cream added, the reaction was about the same as for processed cheese, but, if considerable cream had been added, the reaction becomes more nearly alkaline. As has been shown in processed cheese[2] the reaction is important in connection with the keeping quality of the cheese, a cheese with a reaction above pH 6.3 was more apt to spoil than one having a reaction of 6.2 or less, preferably about pH 5.8 to 6.1. This was demonstrated very clearly in the trouble involved in keeping samples of a cheese spread from spoiling. Examination of the fresh samples revealed a reaction of pH 6.2 to 6.4 with a majority nearer the latter figure. Addition of citric acid sufficient to reduce the reaction to pH 5.9 to 6.05 had no noticeable effect on the flavor and the change in reaction was enough to double or triple the length of time that the cheese spread could be kept in an incubator without spoiling. The reaction figures given are for Cheddar or American Cheese and should not be taken as indicative of the reaction to be found in other varieties. A limited amount of work done on Roquefort and Limburger indicated that these cheese spreads had a reaction between pH 6.4 and 6.7. However, no difficulties were experienced with their keeping qualities. This is probably due to the characteristic fermentations involved in the ripening of these cheeses and the types of organisms that predominate in them.

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[2] Templeton, Hugh L., and Sommer, H. H. JOURNAL OF DAIRY SCIENCE, 13: 303. May, 1930.

CALCULATIONS

Before making any calculations of the amounts of cheese and cream to be used in making a cheese spread it is necessary to decide upon the weight of condensed whey, whey powder or skim milk powder that is to be used, the amount of emulsifying salt, and also the weight of common salt that will be added. The percentage of moisture in each of these substances should be known. With the amounts of these ingredients fixed the general equations for the amounts of cheese and cream to be used, may be stated as:

$aX + bY =$ weight of fat desired

$cX + dY =$ weight of solids-not-fat to be obtained from the cream and cheese

In the above equation X is the weight of cheese having (a) per cent of fat and (c) per cent of solids-not-fat, and Y is the weight of cream with (b) per cent of fat and (d) per cent of solids-not-fat. After the desired fat and solids have been obtained, water may be added to make up the remaining weight and supply any needed moisture for the finished product. Two examples will be given to illustrate the use of these equations.

We desire to make a cheese spread having 26 per cent of fat and 42 per cent moisture. There is available whey powder with 3 per cent moisture, sodium citrate (U.S.P. VIII) containing 27.75 per cent water of crystallization, cheese with 32 per cent fat and 37 per cent moisture, and cream testing 40.0 per cent fat and 5.28 per cent solids-not-fat (calc. from 8.8 per cent solids-not-fat in the whole milk). In the cheese spread we want to use 10.0 per cent of whey powder, 2.0 per cent sodium citrate, and 0.5 per cent common salt. The first step is to make a skeleton table and insert such values as are available:

	FAT LBS.	SOLIDS-NOT-FAT. LBS.	WATER LBS.
10.0 lbs. whey powder	0.00	9.70	0.30
2.0 lbs. sodium citrate	0.00	1.45	0.55
0.5 lbs. salt	0.00	0.50	0.00
lbs. cheese			
lbs. cream			
lbs. water			
100.0 Total	26.00	32.00	42.00

From the above table it is evident that the cheese and cream must furnish the 26.00 pounds of fat and 20.33 pounds of solids-not-fat (32.00 − 9.70 − 1.45 − .50). Substituting the known values in the general equations:

$$.32X + .40Y = 26.00$$
$$.31X + .0528Y = 20.35$$

Multiplying by 100 to remove all but one of the decimals:

$$32X + 40Y = 2600$$
$$31X + 5.28Y = 2035$$

By cross multiplication and subtraction it is possible to eliminate one of the unknowns and solve for the other. By this process Y was found to be 14.5 (calculated to the nearest tenth), and when this value was substituted in the first equation, X was found to be 63.1. Therefore, we must use 63.1 pounds of cheese which will supply 20.19 pounds of fat, 19.56 pounds of S. N. F. and 23.35 pounds of water; 14.5 pounds of cream will furnish 5.80 pounds of fat, 0.77 pounds of S. N. F. and 7.93 pounds of water. Summing up we have a total weight of 90.1 pounds containing 25.99 pounds fat, 31.98 pounds S. N. F., and 32.13 pounds of water. The addition of 9.9 pounds of water will give the total weight of 100 pounds making the moisture content 42.03 which is very close to that originally desired. In practice it is wise to add an excess of water to make up for any losses due to evaporation during the heating process.

As a second example let us take a case where the fat content of the cheese spread will be higher and the moisture lower. Because the latter fact the amount of whey powder to be used will have to be reduced to 9.0 pounds in order to be sure that there will be no crystallization of the lactose. The fat content desired will be 32.00 per cent with 38.0 per cent moisture. The ingredients will be the same except the cheese contains 33.0 per cent fat and 35.8 per cent water, while the cream tests 42.5 per cent fat with 5.1 per cent solids-not-fat. Tabulating the figures available:

	FAT LBS.	S. N. F. LBS.	WATER LBS.
9.0 lbs. whey powder	0.00	8.73	0.27
2.0 lbs. sodium citrate	0.00	1.45	0.55
0.5 lbs. salt	0.00	0.50	0.00
lbs. cheese			
lbs. cream			
lbs. water			
100.0 lbs.	32.00	30.00	38.00

In this case the cheese and cream must furnish 32.0 pounds of fat and 19.32 pounds of solids-not-fat. Making the necessary substitutions we get the equations:

$$.33X + .425Y = 32.00$$
$$.315X + .051Y = 19.35$$

Proceeding as before and eliminating the X we find that Y equals 31.7 or the number of pounds of cream while X is found to be 56.2 or the number of pounds of cheese. This weight of cream will supply 13.46 pounds of fat, 1.62 pounds of S. N. F. and 16.62 pounds of water; the cheese furnishes 18.54 pounds of fat, 17.70 pounds of S. N. F. and 19.96 pounds of water. Summing up we have a total of 99.4 pounds containing 32.00 pounds of fat, 30.00 pounds of S. N. F. and 37.40 pounds of moisture. The addition of 0.6 pounds of water is therefore all that is necessary to give the desired composition.

SUMMARY

1. The desirable spreading qualities of cheese spreads may be due to the moisture content or the fat content of the product or a combination of these factors.
2. High moisture with relatively low fat content gives a mealy texture; as the fat is increased, the consistency becomes like that of butter.
3. High fat and high moisture results in a weak bodied spread that does not give a very pleasing appearance when removed from the container.
4. In making cheese spreads it is essential to heat to a higher temperature because of the high moisture content, less acid reaction, and the presence of putrefactive organisms (if cream is used).
5. The reaction of the cheese spread affects its keeping quality; reactions of pH 5.8 to 6.1 were found to be very satisfactory.
6. A method for the calculation of the amounts of the different ingredients to be used has been worked out and examples given of its application.

The authors wish to express their thanks to Mr. Harry Klueter and Dr. E. O. Huebner for supplying part of the data used in the table.

THE INFLUENCE OF NON-STRIPPING ON THE BACTERIAL COUNT OF MACHINE-DRAWN MILK[1]

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Two practises have developed which make timely a consideration of the influence of non-stripping of cows on the bacterial count of machine-drawn milk. In the first place the bacterial count of milk is used extensively in milk control work as a measure of the cleanliness and desirability of the product. While the types of bacteria present should receive more consideration perhaps than commonly accorded them, yet it is true that large numbers of bacteria are often associated with carelessness in producing and handling the milk. For this reason, the bacterial count serves a useful purpose in giving some indication of the quality of the milk.

If the bacterial count is to be used as an indication of quality and hence in determining to some extent the price received, it becomes important to consider production practises which may affect the number of bacteria present.

Then, in the second place, another practise is introduced for consideration. The returns from the milk obtained by stripping, following the use of a machine, are small in proportion to the extra time and inconvenience involved in doing the stripping.

While most investigators have expressed the belief that stripping is necessary, the practise has been discontinued on some dairy farms with seemingly no deleterious effects. Dairymen producing Certified and other high quality milk, as evidenced by a low bacterial count, are at once confronted with the question as to whether or not non-stripping will have a tendency to increase the bacterial count of the milk produced. A survey of the literature revealed no investigations bearing directly on the problem. Heineman[2] reported work by Lux in which he found an increasing count in samples of milk drawn at increasing intervals of time varying from two hours to twenty-four hours between milkings. This was interpreted as indicating bacterial multiplication in the udder. Stocking[3] found an in-

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[1] The data upon which this paper is based were taken from a thesis presented by G. C. Wallis in partial fulfillment of the requirements for the degree of M.S., University of Minnesota.

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[2] Heinemann, Paul G. Milk, pp. 267–314. W. B. Saunders Co., Philadelphia. 1919.

[3] Stocking, W. A., Jr. Quality of milk affected by common dairy practises. Conn. (Storr's) Agr. Exp. Sta. Bul. 42: 66–90. 1906.

crease in the bacterial count from an average of 6,542 to 11,324 when strippings were left in the udder at the previous milking under hand milking conditions.

The work reported in this article was planned for the purpose of securing some more definite data concerning any change that might occur in the bacterial counts of milk when stripping was not practised.

EXPERIMENTAL

Two lines of approach were followed in obtaining information on this problem. The milking machine as a factor contributing to the bacterial count of the milk was eliminated in the first plan. Six animals were used in this part of the experiment. The milking machine was stopped after it had obtained approximately one-half of the milk. A sample of the middle milk was then drawn separately from each quarter by hand under aseptic conditions. The samples were taken to the laboratory immediately for plating. Samples were obtained in this way from each animal in a two day preliminary period in which stripping was practised regularly. For the next two days stripping was discontinued and samples taken as before. Following this, stripping was again practised and samples taken for two days in a check period. The teats were sealed with collodion between milkings in all of these trials.

The samples were plated in duplicate in at least two, and usually three dilutions. Beef infusion agar was used. The plates were incubated for two days at 37° C. and all plates counted. The most representative dilution was then selected and used as the count of that sample. Representative results obtained from two of the animals used in this work are presented in table 1.

It was conceivable that the foremilk might contain large numbers of bacteria when the strippings were not removed at the previous milking. If the contamination of the foremilk was heavy the bacterial count of the whole milking might be increased by a measurable amount. This condition would not be detected by sampling the middle milk as outlined above. Consequently, the second approach to the problem was designed to cover this point. Two cows were used for this trial. The entire milking was drawn into a specially sterilized milking machine and a sample obtained from this to be used in plating. During the progress of the investigation a check was made to ascertain the bacteriological condition of the milking machine. It was found that the method of cleaning and sterilizing employed was maintaining it in a satisfactory condition.

During the first six day trial with each animal the teats were sealed with collodion between milkings to prevent a variable contamination from this source. The six day trial consisted of two days in which the cows were stripped, followed by two in which stripping was not practised, then two

TABLE 1

The effect of non-stripping on the bacterial count of aseptically drawn milk as measured by sampling the middle milk

DATE	BACTERIAL COUNTS			
	Right fore quarter	Left fore quarter	Left hind quarter	Right hind quarter
	per cc.	*per cc.*	*per cc.*	*per cc.*
		Cow 155		
Stripped previously				
12–26–30 p.m.	220	25	75	40
12–27–30 a.m.	70	20	75	35
p.m.	500	30	25	25
12–28–30 a.m.	1845	30	50	10
p.m.	116	197	24	7
Not stripped previously				
12–29–30 a.m.	365	16	50	27
p.m.	615	6	60	20
12–30–30 a.m.	125	21	17	6
p.m.	173	15	11	7
Stripped previously				
12–31–30 a.m.	380	6	9	7
p.m.	20	10	17	9
1–1–31 a.m.	121	7	27	12
p.m.	1085	11	148	9
		Cow 396		
Stripped previously				
1–2–31 a.m.	19,000	100	10	16
p.m.	6,250	18	15	19
1–3–31 a.m.	9,150	6	7	12
p.m.	3,150	2	13	11
Not stripped previously				
1–4–31 a.m.	12,300	10	24	48
p.m.	3,300	10	19	16
1–5–31 a.m.	4,700	8	18	21
p.m.	6,100	21	12	13
Stripped previously				
1–6–31 a.m.	3,850	12	27	20
p.m.	5,900	141	79	277
1–7–31 a.m.	6,850	295	198	185
p.m.	4,900	222	26	33

days of stripping again, similar to the plan in the first experiment. Each animal was then used in an eight day trial consisting of alternating two-day periods of stripping and non-stripping in which the teats were not sealed between milkings. In both parts of the work the udders were carefully washed with a hypochlorite solution and dried before starting the milking. Other necessary precautions were taken to obtain the sample of milk aseptically. The same bacteriological methods were employed as previously explained. The results obtained in these trials are given in table 2.

Discussion

The data were carefully analyzed by a study of the trend of the counts obtained from period to period for each animal by quarters. The data for all the animals have not been included but it may be noted in the repre-

TABLE 2

The effect of non-stripping on the bacterial count of aseptically drawn milk as measured by a sample of the whole milking

cow 542			cow 382		
Date		Bacterial count whole milking	Date		Bacterial count whole milking
		per cc.			*per cc.*
		Teats sealed			
Stripped previously					
2–11–31	a.m.	10,450	3–17–31	a.m.	105
	p.m.	5,050		p.m.	55
2–12–31	a.m.	48,000	3–18–31	a.m.	160
	p.m.	107,500		p.m.	40
Not stripped previously					
2–13–31	a.m.	167,500	3–19–31	a.m.	125
	p.m.	181,000		p.m.	105
2–14–31	a.m.	101,000	3–20–31	a.m.	45
	p.m.	21,350		p.m.	25
Stripped previously					
2–15–31	a.m.	9,200	3–21–31	a.m.	170
	p.m.	5,550		p.m.	360
2–16–31	a.m.	4,900	3–22–31	a.m.	490
	p.m.	5,150		p.m.	305
		Teats open			
Stripped previously					
2–17–31	a.m.	3,700	3–23–31	a.m.	1745
	p.m.	2,690		p.m.	850
2–18–31	a.m.	2,470	3–24–31	a.m.	515
	p.m.	7,750		p.m.	435
Not stripped previously					
2–19–31	a.m.	15,100	3–25–31	a.m.	935
	p.m.	28,300		p.m.	755
2–20–31	a.m.	64,000	3–26–31	a.m.	920
	p.m.	76,000		p.m.	3810
Stripped previously					
2–21–31	a.m.	35,500	3–27–31	a.m.	1660
	p.m.	11,850		p.m.	1040
2–22–31	a.m.	21,750	3–28–31	a.m.	450
	p.m.	27,250		p.m.	670
Not stripped previously					
2–23–31	a.m.	7,950	3–29–31	a.m.	8260
	p.m.	18,500		p.m.	725
2–24–31	a.m.	44,000	3–30–31	a.m.	915
	p.m.	78,500		p.m.	1870

sentative results shown in table 1 that the two highest counts for the right fore quarter of cow 155 occurred during the stripping periods. There is no abnormal fluctuation during the non-stripping period for any of the quarters. Referring to the last part of table 1, we have no indication that non-stripping tended to increase the counts obtained from cow 396. In fact, the counts are somewhat higher in the last two-day period when stripping was practised regularly. With cow 542 (data not published) the right fore quarter and the left hind quarter had considerably higher counts than the other two. A slight indication of garget was noted in the right fore quarter but the onset was during the preliminary period when stripping was practised, and by the end of the non-stripping period the disturbance had subsided, so the cause can not be attributed to the non-stripping procedure. In the remaining two quarters the counts were somewhat lower during the non-stripping period. A careful study of the data from all six of the cows reveals nothing but normal fluctuations and gives no indication of an increase in the bacterial counts during the non-stripping periods as measured by aseptically drawn samples of the middle milk.

Referring to table 2 giving the data obtained when samples of the whole milking were used, we find practically the same situation. When the teats were sealed on cow 542, the counts were ascending somewhat during the preliminary stripping period. The peak was reached at the second milking during the non-stripping period and by the end of this period the count was relatively low again. In the trials with the teats unsealed the fluctuations from period to period show no decided changes, a count somewhat higher than the rest appearing in each of the non-stripping periods, however.

The results with cow 382 also given in table 2 show some of the lowest counts in the trials with the teats sealed to be in the non-stripping period with a tendency toward higher counts during the last period when stripping was practised. In the first non-stripping period when the teats were left open, the first three counts are on approximately the same level while the last count is somewhat higher. The condition is similar in the second non-stripping period except it is the first count that is a little higher. By studying the data carefully in this manner no conclusive evidence is found to indicate an increase in the bacterial count when the strippings are not removed.

Distribution curves were made as a further step in analyzing the data obtained. A frequency distribution was made of all the bacterial counts obtained during the stripping periods in the first part of the experiment where the middle milk was sampled. These were converted into percentages for each class interval for purposes of comparison. All bacterial counts obtained in the non-stripping periods of all six animals were treated in the same way. The data obtained were plotted as shown in figure 1. The solid line represents the distribution of bacterial counts obtained in the stripping periods and the broken line, those of the non-stripping periods. A study of the graph indicates that there was a somewhat higher percentage of the lower counts during the non-stripping periods. Likewise, the percentage of higher counts is less during the non-stripping periods. According to this analysis the results are somewhat more favorable to the non-stripping periods.

A graph was made in a similar way to represent the data obtained in the stripping and non-stripping periods in the second part of the experiment when the whole milking was sampled. Figure 2 shows the results obtained. The solid line again represents the stripping periods and the

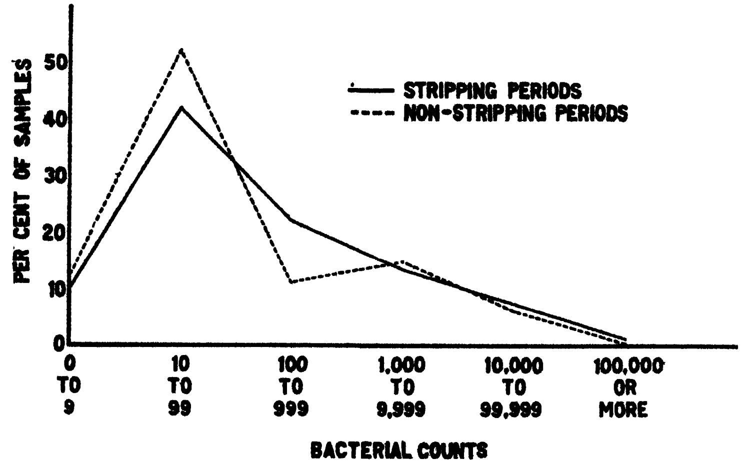

FIG. 1. THE DISTRIBUTION OF BACTERIAL COUNTS AS OBTAINED FROM SAMPLES OF THE MIDDLE MILK DURING STRIPPING AND NON-STRIPPING PERIODS. (DATA BASED ON 296 SAMPLES)

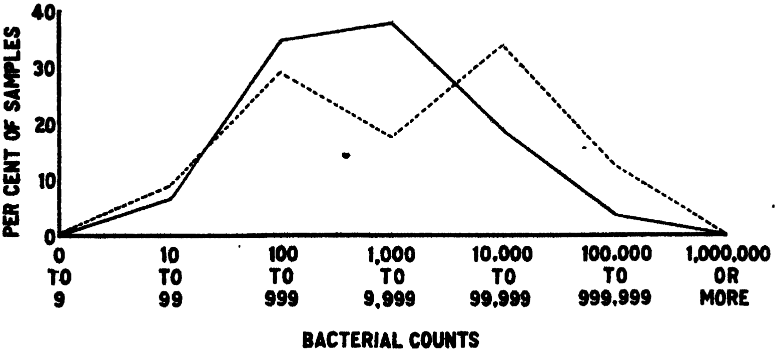

FIG. 2. THE DISTRIBUTION OF BACTERIAL COUNTS AS OBTAINED FROM SAMPLES OF THE WHOLE MILKING DURING STRIPPING AND NON-STRIPPING PERIODS. (DATA BASED ON 56 SAMPLES)

broken line, the non-stripping periods. The percentage of lower counts favors the non-stripping periods only slightly in this case. The two lines then follow each fairly closely until the higher counts are reached when the broken line shows some irregularity and indicates a tendency for a slightly higher percentage of the higher counts in the non-stripping periods. Considering all the factors, the results as indicated in this graph are slightly in favor of the stripping periods. While the amount of data in this second method of investigation is not as large as might be desired, from which to draw a specific generalization, still, sufficient work has been done to indicate what might be expected when stripping is discontinued. A careful study of the data obtained shows no positive evidence that leaving strippings in the udder will increase the bacterial counts of subsequent milkings, as measured in alternating two day trials. If further work should follow the tendency indicated in figure 2 rather than.show normal fluctuations similar in the stripping and non-stripping periods, the present indications are that the increase in bacterial counts from non-stripping would, at least, be slight. The results when sampling the middle milk are slightly in favor of the non-stripping period as shown in figure 1, while the results when sampling the whole milking are slightly in favor of the stripping period, as shown in figure 2, so that the variations noted in the data presented would seem to be more logically explained as normal fluctuations.

A few other interesting observations may be made from the data obtained in this investigation. In agreement with many previous investigations, the bacterial counts of the different quarters of the udder are independent of each other. Some quarters may have high counts while others of the same animal run consistently much lower, as with right fore quarter of 396, and 155 noted in table 1. Similar conditions were noted in the unpublished data for 542 and 402. The counts may be increasing in some quarters while decreasing in others.

Likewise, there is no evidence of a correlation between the thoroughness of machine milking and the normal bacterial count of the cow. Cow number 542 milked out poorly and showed a high count, while 547 also retained considerable strippings but had a low count. On the other hand cow 155 milked out well and had a low count, while 409 milked out equally well but gave a much higher count.

It might also be expected that cows milking out poorly with the machine would have a greater tendency toward increased bacterial counts in non-stripping periods, but this was not found to be the case in the present investigation. The results were negative with both types of animals.

The effect of retained strippings on the bacterial content of milk from high count cows can be compared with normally low count animals. Again, no difference appeared. As measured by a sample of the middle milk there was no increase in the bacterial count during the non-stripping period with either the high or low count type.

Some idea may also be gained as to the effect of sealing the teats on the resulting bacterial count. According to information in table 2, for cow 542, the counts run somewhat higher where the teats were sealed, and the lowest counts of the entire series are in the first part of the period when the teats were left open. With cow 382, the opposite is the case. The lowest counts were obtained when the teats were sealed. The fluctuations are approximately as violent in one case as the other so no definite claims can be made for or against the sealing of the teats.

CONCLUSIONS

From a study of the data presented the following conclusions seem justified.

1. There is no positive evidence that leaving strippings in the udder will increase the bacterial count of the subsequent milkings, as measured in alternating two-day periods of stripping and non-stripping. The variations noted would seem to be more properly explained as normal fluctuations.

2. The bacterial counts of the different quarters of the same udder are independent of each other.

3. There is no correlation between the thoroughness of the machine milking and the normal bacterial count of aseptically drawn milk.

4. The effect of non-stripping on the bacterial count of the milk is negative with cows that milk out poorly, as well as with those that milk out well with the machine. The same is also true for cows normally of the high count type and those of the low count type.

5. Sealing the teats with collodion between milkings seemed to have no noticeable effect in reducing the bacterial counts or preventing the relatively wide fluctuations.

THE BEHAVIOR OF COWS ON ALFALFA HAY AS THE SOLE ROUGHAGE AND ON ALFALFA AND TIMOTHY COMBINED

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INTRODUCTION

For the past ten years the Beltsville Station of the Bureau of Dairy Industry has been carrying out work directed toward discovering methods of feeding and treatment for dairy cows which could be easily controlled experimentally and which would keep cows for an indefinitely long period in good health and at optimum or nearly optimum milk-producing and reproductive capacity. This problem is by no means solved, but certain cows have done moderately well on fairly uniform rations for periods of about five years, and some important landmarks in the field of experimentation seem to be outlining themselves. It is worth-while, therefore, to give a partial account of the results, emphasizing particularly certain aspects of them which bear on the question of the importance of hay in the dairy ration.

OUTLINE OF METHODS OF FEEDING AND TREATMENT

The cows are, of course, kept entirely off pasture. The rations fed consisted at first of grain combined with either alfalfa or timothy hay and corn silage. In the year 1922, however, an epidemic of scouring and digestive disturbance broke out in the experimental herd, which, it was thought, might be due to some unusual molds or bacteria in the corn silage. From that time on a number of cows were fed on grain with either alfalfa or timothy hay as the sole roughage, although certain others continued to receive one of the two kinds of hay and silage in addition.

When the cows were dry, it was aimed to feed them ten per cent more total digestible nutrients than they required according to the Savage standard (6, p. 133). After they calved, it was often found that for some time they would not eat as much as they required according to this standard. It was aimed, however, to feed as much as they required for the first two clear calendar months after calving. When this could not be accomplished, they were fed as much as they would eat. After they had been milking for two clear calendar months, it was aimed to feed them ten per cent more than they required according to the Savage standard, and to continue this surplus feeding until the next calf was born. As a matter of fact, however, the cows were not infrequently fed somewhat more than the above schedule

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calls for. The rations used were high in protein, so that when the cows ate ten per cent more total digestible nutrients than they required according to the Savage standard they usually consumed from thirty to seventy per cent more digestible protein than they required.

The cows which received silage were fed 9 kilograms of it or less daily, as much hay as they would eat, and enough grain to bring their consumption of total digestible nutrients up to the schedule which has just been given. In the case of those fed only grain and hay, it has been the aim to feed equal quantities by weight of grain and hay. When, however, it was found that the cows would not eat enough on this basis to supply what they required to conform to the schedule of feeding, they were given as much hay as they would eat, and enough grain to conform to the schedule. The grain mixtures used for the cows for which data are given in this paper were numbers 40, 50, 60, and 75. Their composition is given in table 1.

TABLE 1

*Composition of grain mixtures**

Grain 40	
Corn meal	50
Wheat bran	50
Salt	1

Grain 50	
Corn meal	40
Wheat bran	30
Cottonseed meal	20
Linseed meal	10
Salt	1

Grain 60	
Corn meal	40
Wheat bran	30
Soybean meal	20
Linseed meal	10
Salt	1

Grain 75	
Corn meal	40
Wheat bran	40
Linseed meal	20
Salt	1

* The corn meal used throughout the experiments was yellow. The cottonseed, linseed, and soybean meals had their fat extracted by the old process, that is, without fat solvents.

In the earlier stages of the experiments, from 1920 until 1924, the quality of the hay used was not carefully controlled and a number of the experimental animals got either alfalfa or timothy hay which was of mediocre and sometimes probably of rather poor quality. After 1924, however, and in all of the experiments for which figures are given in this paper, it was aimed to feed only hay of high quality. The alfalfa hay used was mostly of U. S. No. 1 grade (8). For short periods, sometimes, it was found necessary to feed hay of a high No. 2 grade, but these periods were balanced by others in which extra leafy and extra green alfalfa was fed, so that all the alfalfa fed may be regarded as of No. 1 grade on the average. In the case of timothy hay, it is sometimes practically impossible to get the U. S. No. 1 grade (8). At such times a high grade No. 2 hay was fed, but most of the timothy fed was of the No. 1 grade, and all of it, of good quality.

When indoors the cows are kept in stanchions. They are turned out in a lot where there is no grass for at least two half-hour periods every day to get water. Through most of the summer, and in good weather in winter, they spend a considerable portion of the day in this lot.

The cows are fed individually in smooth cement mangers, so that any food left by them can be conveniently removed and weighed. All the daily portions of grain, hay and silage offered to them are weighed and if, after a certain interval, these are not consumed the portions left are removed and weighed. Daily records of all the feed consumed have been kept as well as of all the milk yielded. In the early stages of the experiments the fat content of the milk from each cow was determined by the Babcock test on two days each month. More recently this test has been made three times a month, each time on two successive days.

For convenience many of the records have been kept on the basis of calendar months. At the end of each calendar month the daily milk yields of each cow are added up and the sum for the month is recorded. The fat yield for the month is calculated from this sum by means of the average test as determined from the Babcock tests. The weights of all feeds consumed are also added up at the end of the month and the sums recorded.

Each cow is weighed on three successive days at the beginning of each month, and the average weight for the three day period is recorded.

From the body weights of the cows and the monthly milk and fat yields, their requirements for digestible protein and total digestible nutrients according to the Savage standard are calculated. Their consumption of digestible protein and total digestible nutrients for the month is also calculated from the consumption of the various feeds, using the average figures for the digestible protein and total digestible nutrients of dairy feeds which are given by Henry and Morrison (6, p. 653). The requirement and consumption are recorded and compared, and used as a guide in feeding the cows from month to month according to the foregoing schedule.

The heifers are bred for the first time at the age of twenty-one months. After they calve, the animals are not bred until they have been milking for six clear calendar months. If the calving date falls less than seven days before the beginning of the succeeding calendar month, the next month after this is generally counted as the first clear calendar month of lactation. The cows are dried off after they have been milking for twelve clear calendar months or more, it being the aim to make the dry period about one-quarter the length of the milking period. If they become pregnant as the result of the first breeding, the milking period is only a little over twelve months; if there is delay about getting them with calf, it may be considerably longer.

EXPERIMENTAL RESULTS

It was observed fairly early in the experiments that the cows which got timothy hay of mediocre quality, whether or not combined with corn silage, would not eat large quantities of it, and that after a time they showed a strong desire to steal alfalfa from their neighbors. Sometimes they ate only very small quantities of hay and would then show a general loss of appetite, reduction in milk yield, and signs of general sickness. They sometimes recovered from this condition when alfalfa was substituted for the timothy hay.

Five cows which were fed for long periods on timothy of mediocre quality, either as the sole roughage or combined with corn silage, lasted on the average only a little more than a year and a half, and in no case for as much as three years. They either died of illnesses, which were variously diagnosed, or failed to become pregnant, though bred repeatedly.

Five cows, on the other hand, have now been on alfalfa hay either as the sole roughage or combined with other roughages for periods of over five years. These are cows 71, 84, 458, 470, and 482. Cows 71 and 84 are grade Guernseys; cows 458, 470, and 482, pure-bred Jerseys. It is the history of these five cows which forms the particular subject of this article.

One of these, cow 84, has been on grain, alfalfa, and silage since November 1, 1924, and is still doing fairly well at present (March 4, 1931) at the age of over thirteen years.[1] A second one, cow 458, has been on grain with alfalfa as the sole roughage since November 1, 1926,[2] and is still doing well at present at the age of over nine years.[3] The other three, namely, cows 71, 470, and 482, were fed on grain with alfalfa as the sole roughage for periods of from two years and nine months to three years and three months. It was noticed that these cows as well as others which were kept for long periods on alfalfa as the sole roughage, or on alfalfa combined with corn silage, showed a strong desire to steal timothy hay from their neighbors. This suggested that a ration in which alfalfa was the sole hay fed might not be altogether ideal for dairy cows, and led to the experiments on feeding alfalfa and timothy together which form the subject of this article.

One of the three cows mentioned above, No. 482, did not eat hay well during her lactation period of 1926–27, and left small quantities even during the subsequent dry period. She aborted September 24, 1927, after a dry period of about four months, but six weeks before the calf was due. She did not show any very marked loss of appetite at this time, but in view of the observations mentioned above it was suggested by one of us (H. T. C.) that she be given some timothy hay along with the alfalfa. The suggestion was acceded to with some reluctance by the other author, as it seemed doubtful whether it was wise to break the continuity of an experiment on feeding grain and alfalfa which had already continued for nearly three years. However, the cow was offered 1.8 kilograms of timothy hay daily beginning the fourth day after calving and continuing for some weeks thereafter. Some of the details of her behavior for the first six weeks after calving are given in table 2. In spite of the combined timothy and alfalfa feeding, she was badly off feed in the first and second weeks after calving. She showed a decided preference for timothy over alfalfa, however, not only for the six weeks after calving, for which data are given in table 2, but also for the second, third, and fourth months after calving. After that she returned to a preference for alfalfa hay. Her milk yield, in spite of the abortion, was decidedly higher than it had been in either of her two previous lactations.

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[1] Born Oct. 11, 1917.

[2] She was on grain with alfalfa and silage for some time before this.

[3] Born June 20, 1921.

Cow 482 had been on grain, timothy, and alfalfa for about a year when cow 470 calved on October 1, 1928. Data regarding her behavior for two weeks before and six weeks after calving are given in table 2. She showed no particular distaste for alfalfa in the lactation period preceding this calving, and her appetite was good in her dry period and up to the time of calving. In the first two weeks after calving, however, she was very badly off feed, eating less than half of the very moderate amounts of hay and grain offered to her. On three days scattered through this period she ate no grain at all, and on two days no alfalfa hay. On the last day of the second week, she was offered 2.5 kilograms of timothy hay which she ate up clean, at the same time refusing completely the 2.5 kilograms of alfalfa which were offered to her. From this time on she was offered gradually increasing quantities of both timothy and alfalfa and ate some of each but showed a decided preference for the timothy. Table 2 shows how her appetite and milk yield improved and how definitely she preferred timothy to alfalfa during the third, fourth, fifth, and sixth weeks after calving, when she was fed grain combined with the two kinds of hay. Her preference for timothy lasted all through the subsequent lactation period, though she ate large quantities of both kinds of hay.

Cow 71 calved on December 1, 1928. Some details of her behavior are shown in table 2. She showed some distaste for alfalfa hay for a week before and for three or four weeks after calving, and the question of giving her timothy in addition to alfalfa was considered. Decision was delayed on account of the absence of one of the authors from the experiment station, but she was given three kilograms of timothy on the last day of the eighth week after calving. By this time she was eating satisfactory quantities of alfalfa hay but was still refusing considerable portions of the grain which she required according to the Savage Standard for her maintenance and milk yield. In the ninth week after calving and subsequently she was offered gradually increasing quantities of both alfalfa and timothy hay.

TABLE 2

Food consumption and milk yield of cows 482, 470, and 71, for periods immediately before and after calving.

PERIOD	DAILY AVERAGE									CONSUMPTION[1] AS PER CENT OF REQUIREMENT[1]
	GRAIN		ALFALFA		TIMOTHY		TOTAL HAY EATEN	MILK YIELD	TOTAL DIGESTIBLE NUTRIENTS REQUIRED	
	Offered	Eaten	Offered	Eaten	Offered	Eaten				
	kgs.	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	
Cow 482[2]										
Week before calving										
Second	3.6	3.6	3.5	3.1	0	0	3.1		4.24	98.6
First	3.6	3.5	3.5	3.2	0	0	3.2		4.29	97.0
Week after calving										
First	5.0	4.0	3.7	2.4	1.0	0.9	3.3	8.7	7.99	56.8
Second	5.6	2.0	3.6	0.5	1.8	0.6	1.1	9.4	8.21	25.1
Third	5.9	5.5	3.6	2.7	1.8	1.8	4.5	10.3	8.59	74.6
Fourth	6.6	4.3	3.6	2.1	1.8	1.7	3.8	12.4	9.52	54.1
Fifth	7.0	7.0	3.6	2.1	1.8	1.8	3.9	13.7	10.09	71.8
Sixth	8.6	8.6	3.6	2.2	2.5	2.5	4.7	15.4	10.75	82.2
Cow 470[3]										
Week before calving										
Second	3.6	3.6	3.5	3.4	0	0	3.4		3.58	124.9
First	3.6	3.6	3.5	3.4	0	0	3.4		3.63	123.1
Week after calving										
First	6.5[4]	3.6	3.8	2.6	0	0	2.6	9.9	8.34	33.8
Second	4.6	1.6	4.6	1.5	0.4	0.4	1.9	9.3	7.99	27.2
Third	4.7	3.1	2.5	1.6	3.2	3.1	4.7	9.6	8.09	57.7
Fourth	5.3	4.9	2.6	2.0	3.0	3.0	5.0	12.9	9.66	64.1

[1] Of total digestible nutrients.
[2] Born 6/3/22; calved 9/24/27.
[3] Born 9/17/21; calved 10/1/28.
[4] On account of unwillingness to eat grain No. 60, cow 470 was offered considerable quantities of bran during this week.

TABLE 2—*Continued*

Food consumption and milk yield of cows 482, 470, and 71, for periods immediately before and after calving.

PERIOD	DAILY AVERAGE									CONSUMPTION[1] AS PER CENT OF REQUIREMENT[1]
	GRAIN		ALFALFA		TIMOTHY		TOTAL HAY EATEN	MILK YIELD	TOTAL DIGESTIBLE NUTRIENTS REQUIRED	
	Offered	Eaten	Offered	Eaten	Offered	Eaten				
	kgs.	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	
Cow 470[3]										
Fifth	5.9	5.9	3.0	2.8	3.1	3.1	5.9	14.5	9.97	74.1
Sixth	6.4	6.4	3.3	3.1	4.1	4.1	7.2	15.4	10.05	83.8
Cow 71[5]										
Week before calving										
Second	4.0	1.9	3.5	3.2	0	0	3.2		4.41	68.7
First	4.0	3.3	3.5	1.8	0	0	1.8		4.43	75.2
Week after calving										
First	5.4	4.9	4.2	3.7	0	0	3.7	10.4	8.23	66.5
Second	7.9	4.5	5.4	4.5	0	0	4.5	13.6	9.47	59.0
Third	8.0	5.0	6.5	5.6	0	0	5.6	14.0	9.58	68.2
Fourth	8.0	4.9	6.6	5.8	0	0	5.8	15.7	10.25	63.9
Fifth	8.0	4.3	7.0	6.9	0	0	6.9	15.8	9.97	67.1
Sixth	8.0	7.2	7.4	6.2	0	0	6.2	17.6	10.40	81.1
Seventh	8.0	6.1	8.0	7.8	0	0	7.8	17.8	10.48	80.6
Eighth	8.0	5.2	7.6	7.5	0.4	0.4	7.9	16.1	9.84	79.7
Ninth	8.0	7.3	5.1	4.9	4.4	4.2	9.1	16.3	9.84	100.4
Tenth	8.0	8.0	5.5	5.5	5.5	4.4	9.9	16.2	9.70	111.2
Eleventh	8.4	7.8	5.5	5.5	5.2	3.9	9.4	16.9	9.96	104.4

[5] Born 7/15/16; calved 12/1/28.

TABLE 3

Yearly amounts of food eaten, and percentage total digestible nutrients consumed above or below requirements by cows on various rations.

DATES OF PERIODS	KIND OF GRAIN	AMOUNT OF GRAIN EATEN	ALFALFA HAY EATEN	TIMOTHY HAY EATEN	CORN SILAGE EATEN	TOTAL DRY ROUGHAGE EATEN	CONSUMPTION[1] AS PER CENT ON REQUIREMENT[1]
Cow 71	*No.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	*kgs.*	
5/1/26 to 4/30/27	75	2625	2425	0	0	2425	111.1
10/1/27 to 9/30/28	40 & 75[2]	2153	2081	0	0	2081	119.0
1/1/29 to 12/31/29	75	2273	1563	979	0	2542	113.1
Cow 84							
4/1/25 to 3/31/26	50	2054	1921	0	2040	2458[3]	128.9
12/1/26 to 11/30/27	60	2265	1905	0	1560	2315[3]	109.3
4/1/28 to 3/31/29	50	2265	1994	0	1696	2440[3]	107.2
11/1/29 to 10/31/30	50	2214	1997	0	2227	2583[3]	117.0
Cow 458							
4/1/27 to 3/31/28	60	2597	2196	0	0	2196	98.0
7/1/28 to 6/30/29	60	2501	1829	0	0	1829	95.3
Cow 470							
1/1/26 to 12/31/26	75	2891	2483	0	0	2483	107.3
6/1/27 to 5/31/28	60	2797	2293	0	0	2293	105.7
11/1/28 to 10/31/29	60	2648	1324	1567	0	2891	108.0
3/1/30 to 2/28/31	60	3029	1521	1663	0	3184	105.5
Cow 482							
1/1/25 to 12/31/25	50 & 75[4]	1922	1997	0	2104	2550[3]	115.8
5/1/26 to 4/30/27	75	2697	1845	0	0	1845	107.6
10/1/27 to 9/30/28	60	2779	1167	995	0	2162	101.8
2/1/29 to 1/31/30	60	2572	1285	1047	0	2332	104.3

[1] Of total digestible nutrients.
[2] 1101 kg. of No. 40 and 1052 kg. of No. 75.
[3] Weight of alfalfa plus weight of dry matter in the corn silage eaten.
[4] 1767 kg. of No. 50 and 155 kg. of No. 75.

Her appetite improved very markedly and she soon ate more than enough grain and hay combined to cover her requirements. Her milk yield also rose somewhat above what it had been in the eighth week after calving, though it never again reached the rather high level which had been maintained through the sixth and seventh weeks after calving. Unlike cows 470 and 482, however, cow 71 did not show any preference for timothy over alfalfa at any time during the subsequent lactation period. During most of the period she showed a quite definite preference for alfalfa.

Cows 71, 470, and 482 did so well in their first lactation periods on grain with alfalfa and timothy hay combined that they have been continued on these rations, as far as possible, up to the present time. Some details of their performance are given in tables 3 and 4. Similar figures for the performance of cows 84 and 458 are put in for comparison. Cow 458 is a cow of about the same milk-yielding capacity as cow 470, and has been on grain with alfalfa as the sole roughage since November 1, 1926. The figures in tables 3 and 4 represent the food consumption, milk yield, etc., in periods, each of which lasted 365 days.

The first point to which attention should be called is that the cows ate decidedly more hay when they got alfalfa and timothy together than when they got alfalfa alone. In connection with this point the figures in table 3 for hay consumption during whole lactation periods should be supplemented with figures for individual months of lactation. Such figures are given in table 5. The cows usually eat the largest amounts of hay in the early months of lactation, while the milk yield is still large, and it is at this time that the greatest differences appear in their willingness to consume alfalfa as the sole roughage on the one hand and alfalfa and timothy combined on the other. It appears from the figures given in table 5 that cows 71, 470, and 482 consumed about 30 per cent more of the combined hays on the average in their record months than they did of alfalfa alone. Table 5 contains figures for the roughage consumption of cows 84 and 458 as well as of cows 71, 470, and 482. These figures give some further evidence for the view that it is difficult to get cows of the Jersey and Guernsey types to eat as much as 8 kilograms of roughage daily, when the roughage in question consists of alfalfa hay alone. In commenting on the results given in table 5, it may be stated that an effort was always made to get the cows to eat as much roughage as possible.

The figures for milk and fat yield given in table 4 are also interesting. Cow 71 gave much more milk and fat in the first lactation on grain, alfalfa, and timothy than in the previous lactation on alfalfa as the sole roughage. It is true that the milk and fat yields on the combined hays were markedly less than they had been about three years before on alfalfa as the sole roughage, but cow 71 was well over 12 years old when she was first put on the combined hays, and her failure to improve on her best previous record

TABLE 4

Yearly milk and fat yields and total digestible nutrients required by cows on various rations.

DATES OF PERIODS	AGE AT STARTING	RATION	MILK YIELD	FAT YIELD	TOTAL DIGESTIBLE NUTRIENTS REQUIRED
Cow 71			*kgs.*	*kgs.*	*kgs.*
5/1/26 to 4/30/27	9 yrs., 10 mos.	Grain & alfalfa	4101	157	2844
10/1/27 to 9/30/28	11 yrs., 3 mos.	Grain & alfalfa	2055	77	2207
1/1/29 to 12/31/29	12 yrs., 6 mos.	Grain, alfalfa & timothy	3368	132	2594
Cow 84					
4/1/25 to 3/31/26	7 yrs., 6 mos.	Grain, alfalfa, & corn silage	2386	111	2248
12/1/26 to 11/30/27	9 yrs., 2 mos.	Grain, alfalfa, & corn silage	3633	164	2717
4/1/28 to 3/31/29	10 yrs., 6 mos.	Grain, alfalfa, & corn silage	3893	174	2801
11/1/29 to 10/31/30	12 yrs., 1 mo.	Grain, alfalfa, & corn silage	3435	153	2630
Cow 458					
4/1/27 to 3/31/28	5 yrs., 9 mos.	Grain, & alfalfa	4367	246	3157
7/1/28 to 6/30/29	7 yrs.	Grain & alfalfa	3849	215	2973
Cow 470					
1/1/26 to 12/31/26	4 yrs., 3 mos.	Grain & alfalfa	4281	252	3152
6/1/27 to 5/31/28	5 yrs., 8 mos.	Grain & alfalfa	4325	241	3117
11/1/28 to 10/31/29	7 yrs., 1 mo.	Grain, alfalfa, & timothy	4289	246	3169
3/1/30 to 2/28/31	8 yrs., 5 mos.	Grain, alfalfa & timothy	5422	304	3677
Cow 482					
1/1/25 to 12/31/25	2 yrs., 7 mos.	Grain, alfalfa, & corn silage	2434	150	2449
5/1/26 to 4/30/27	3 yrs., 11 mos.	Grain & alfalfa	2951	168	2708
10/1/27 to 9/30/28	5 yrs., 4 mos.	Grain, alfalfa & timothy	3812	216	3117
2/1/29 to 1/31/30	6 yrs., 8 mos.	Grain, alfalfa, & timothy	3445	193	2985

TABLE 5

Largest amounts of dry roughage eaten by cows in calendar months on different rations, and circumstances under which these record amounts of roughage were eaten

COW	DATE	RATION	GRAIN		TOTAL DRY ROUGHAGE[1]		MILK YIELD	CONSUMPTION[2] AS PER CENT OF REQUIREMENT[2]
			Offered	Eaten	Offered	Eaten		
			kgs.	kgs.	kgs.	kgs.	kgs.	
71	Oct., 1926	Grain & alfalfa	279	252	232	228	335	130.7
458	May, 1927	Grain & alfalfa	271	252	248	233	551	94.3
470	March, 1926	Grain & alfalfa	279	279	248	235	443	107.2
482	July, 1926	Grain & alfalfa	267	262	217	182	374	106.5
84	Jan., 1930	Grain, alfalfa & corn silage	248	245	282	279	423	126.7
482	March, 1925	Grain, alfalfa & corn silage	186	186	235	229	250	121.1
71	Feb., 1929	Grain, alfalfa & timothy	235	230	308	266	443	112.3
470	Dec., 1928	Grain, alfalfa & timothy	267	267	383	323	506	110.3
482	March, 1929	Grain, alfalfa & timothy	248	248	281	245	466	99.6

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[1] When the cows were on alfalfa and corn silage the "Total Dry Roughage" eaten is calculated by adding the amount of hay eaten to the amount of silage multiplied by 0.263. This figure represents the dry matter in the silage.

[2] Of total digestible nutrients.

might reasonably be attributed to her age. She is still doing fairly well at the present time (March 4, 1931).

Cow 470 did about as well in her first lactation on the combined hays as in the two previous lactations on alfalfa as the sole roughage, in spite of the fact that she was quite sick at the beginning of the first mentioned lactation and began at this time to show some stiffness in her legs, possibly of a rheumatic nature. In her second lactation on the combined hays she has done much better than she ever did before and better than any of the Jersey cows in the group on these experiments. Her stiffness has become somewhat more marked and she has been kept in a box stall, while in the barn, in her 1930–31 lactation period. Experiments carried out at this station in 1922 and 1923, but still unpublished, have indicated that keeping cows in box-stalls instead of stanchions has a favorable influence on milk and fat yield amounting to less than 5 per cent. In the case of cow 470, it is doubtful whether the favorable influence of the box stall would have offset the unfavorable influence of her gradually increasing stiffness. She is still doing very well at the present time (March 4, 1931).

Cow 482 gave decidedly more milk and fat in the two lactations on the combined hays than in either of the two preceding lactations. She died on August 5, 1930, six months after the end of the second lactation on the combined hays and was found on post mortem examination to have a widespread inflammation of the intestines.

DISCUSSION

The foregoing results seem to indicate that cows do not eat at their best on rations in which either alfalfa or timothy hay is the sole roughage; that combining the two hays increases their appetite and particularly their willingness to eat large quantities of hay; and that the increased hay consumption on the combined roughage is likely to be accompanied by an increased milk and fat yield.

That cows receiving no hay except alfalfa develop a craving for other kinds of hay has been observed by Erf (2), and he states that adding blue grass or timothy hay of good quality to the rations of such cows increases their milk yield. He does not, however, give any figures to show either increased hay consumption or increased milk yield on the combined hays. A study has recently been made of the hay preference of about twenty-five milking cows in the Beltsville herd which had previously been on various rations—some on either alfalfa or timothy as the sole roughage, and some on alfalfa and corn silage. When offered both kinds of hay at the same time, the cows which had previously been on either as the sole roughage for any length of time almost always showed an unmistakable preference for the other. On the whole, there seems a great deal of reason to believe that for some reason or other alfalfa and timothy hay combined make a more suitable roughage for dairy cows than either fed by itself.

In regard to the effects of the combined roughages on milk yield, on the other hand, a good deal of caution should be observed. The results so far obtained at Beltsville are based on work with only three cows. Further experiments designed to confirm or correct them are already under way.

In considering the nutritional background for the observations that have been made, it must be emphasized that the hay fed was all of good quality. The results furnish no guarantee that cows would eat large quantities of combined timothy and alfalfa hay of poor quality, or that the consumption of large quantities of poor hay would improve the milk yield. The effect of different qualities of hay on the appetite and milk yield of cows over long periods is a subject which is in great need of investigation. That hay, and particularly hay of good quality, supplies some unknown dietary essentials which are important in maintaining the health and milk yield of dairy cows has long been evident (1), (3), (4), (5), (7), (9), (10).

SUMMARY

Cows which are fed on grain with either timothy or alfalfa hay as the sole roughage are likely to consume rather small quantities of roughage and sometimes have periods of digestive disturbance and lack of appetite in which they show a very definite distaste for the kind of hay that they have had for some time past. Feeding the other kind of hay under such circumstances has often been followed by fairly rapid recovery from the digestive depression. The continued feeding of high-quality alfalfa and timothy together, under such circumstances that the cows could eat as much of each kind of hay as they wished, has resulted in a decidedly larger consumption of hay and in a larger milk yield than when alfalfa of good quality was the only kind of hay fed.

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RIPENING CHEESE IN A SEALED PACKAGE

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Marketing cheese of the cheddar type is handicapped by unsuitability of the package to modern methods of merchandising.

There is a material loss in weight by evaporation of water during curing, loss from the rind formed by this evaporation, and additional waste in cutting. It is at a disadvantage by comparison with the attractive packages of nearly all food products and because of the inconvenience of cutting portions for each customer. When cut it is in an inconvenient form for carrying or holding. Still another objection to the present form of package is the loss of identity of the maker. It is very difficult for a factory to so brand its output that a reputation for high quality can be established among the consumers. Consequently there has not been the inducement to produce cheese of the high quality that the industry has needed. This is reflected in the low per capita consumption of cheese in this country.

The process requiring melting and mixing with an emulsifier has been successful, except that many of the characteristics of the cheese are destroyed and the appeal to the consumer has come largely through the attractiveness of the package.

These difficulties could be very largely overcome if a method could be devised for canning cheese as it comes from the press. The advantages of a canned cheese are obvious especially if the curd is put into the can from the press, thus eliminating the cost of paraffining and the losses due to shrinkage and the formation of a rind.

In packaging cheese, and especially in canning, two obstacles must be overcome. Cheese is an excellent medium for molds and if it is confined so that the surface does not become dry it will soon be covered with mold colonies. It is scarcely conceivable that sufficient precautions could be taken to completely exclude mold contamination from a product of this nature, but mold growth can be controlled by limiting the oxygen supply. If cheese at any stage of ripening is packed in hermetically sealed cans so

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[1] The author is indebted to his associates, K. J. Matheson, H. L. Wilson, and W. T. Johnson, Jr., whose assistance in making and canning the cheese has made this investigation possible.

Application has been made for public service patents on the methods and devices described in this paper. On account of the many patents on processes of preserving foods, any one considering selling cheese in cans or glass jars should secure the advice of a competent patent attorney.

that there is only a very limited air space, molds will not develop, at least not to an extent to impair the value of the cheese. In a very restricted space mold colonies may begin development, but the oxygen is soon exhausted and growth ceases. These incipient colonies usually disintegrate and disappear.

The second obstacle is due to the evolution of gas by the cheese, which in its normal ripening, produces gas in amounts sufficient to develop considerable pressure. If this gas is confined it will in time swell and burst the package.

Pernot[2] describes experiments in canning cheese in which the cheese was pressed in the cans or transferred from the hoops direct to the cans. Apparently there was no molding and no trouble from swelling except when the cheese was overheated while in a half ripened condition. Doane, while connected with the Dairy Division of the Bureau of Animal Industry, made an extensive investigation of canning cheddar cheese. These experiments were successful in producing a cheese of fine quality and free from mold but such a large percentage of the cans eventually swelled that the method was not considered practicable.

The trouble from swelling may be overcome by providing a valve which will permit the escape of gas from the can without admitting air. A valve which we have found satisfactory for this purpose is shown in Figure 1.

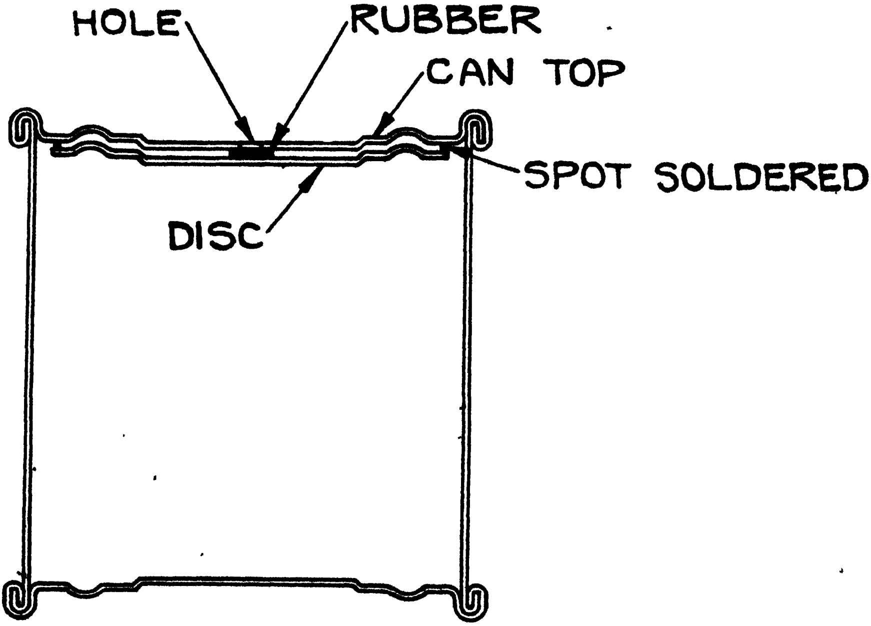

Fig. 1. Cross-section of the top of a can fitted with relief valve.

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[2] E. F. Pernot. Canning Cheese. Bulletin 78. Oregon Agr. Exp. Sta. 1904.

A hole about 1/16 inch in diameter is punched in the top of the can. A seat of thin rubber or other suitable material is held against this opening by a circular tin disk soldered or seamed to hold it in place without completely closing the space between the disk and the top to the passage of gas. This holds the rubber seat firmly against the opening and prevents the ingress of air at all times. If sufficient pressure is developed in the can to bulge the top slightly, the valve is opened and gas escapes until the pressure is relieved.

In an ordinary No. 2½ can, from three to five pounds pressure is required to open the valve. Failure of the valve to open may occur if whey, pressed out of the cheese, works its way into the valve and dries, thus cementing the seat to the top. This will not happen if the cheese is of good quality, and the can is not filled too full and is not exposed to high temperatures.

In the experimental work conducted on a small commercial scale the only serious difficulty encountered has come from defective cans and imperfectly soldered disks. This was overcome by the selection of suitable cans and no difficulty from molding has been experienced in cans free from these defects.

In our later work a modification of the well-known Bunsen valve has been used. A cross-section of this valve is shown in Fig. 2. It consists of a rubber dome projecting through a hole in the can top and cemented to the metal. A cut is made with a thin, sharp pointed knife in such a way that the opening on the outside is near the metal as shown in Fig. 2. Pressure from without closes this opening effectively, while it is opened by relatively low pressure from within. Pure gum rubber has a tendency to stick so that a high pressure is necessary to open, but this may be overcome by putting powdered talc on the knife blade in making the cut. Rubbers containing $CaCO_3$, prepared for us by the Bureau of Standards, have given no trouble in this way. The valve has an advantage over the one shown in Fig. 1 in that its action is independent of the size or shape of the can. We have used it successfully on cans containing 8 pounds of cheese.

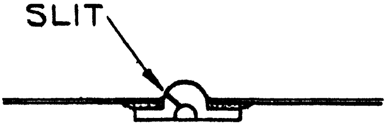

FIG. 2. Modified Bunsen valve.

Only curd of the best quality should be put into cans. Aside from the commercial aspects of selling cheese with an inferior flavor under a special brand, the difficulty of providing for the expansion of the cheese from an abnormal gassy fermentation is obvious. The valve can be expected to take care only of the gas which escapes from the body of the cheese.

Cans for this purpose must seal absolutely tight. A pin hole will admit sufficient air to support abundant mold growth. The so-called packer's can, used for fruit, vegetables, and other food products requiring sterilization, answers this requirement. Lacquered cans should be used to prevent the blackening which occurs in spots when cheese is held in direct contact with tin.

Hoops should be made with an internal diameter 1/8 inch smaller than the internal diameter of the can. This will make a cheese which may be removed from the can without difficulty if the top is entirely removed. If the cheese fits the can closely the flow of gas toward the valve may be prevented and sufficient pressure may develop in the valveless end to cause swelling. A long hoop may be used and the cheese cut into the lengths required to fill the can. If the curd is weighed into the hoop, the operator can, with a little experience, gauge the amount of curd in each hoop to give a cheese which will cut into an exact number of prints without waste.

A can should be selected which will give the required capacity and at the same time have as large a diameter as practicable. Thus, a No. 3 can, which has an internal diameter of 4-1/16 inches, would require a height of 2-15/32 inches to hold a one-pound print, allowing 1/8 inch space between the cheese and the can. Hoops for pressing for this size can should be 3-15/16 inches, internal diameter, and may be 14 inches long. These hoops will be more easily filled than hoops for a No. 2 or 2½ can. The hoops may be filled without bandage, pressed for a short time, removed from the hoops to apply the bandage, and the pressing completed. When the cheese is finally taken from the hoops the bandage should be removed.

Cheese ripens normally in cans but since there is no loss of water there is a tendency toward a softer texture and there is no rind. A high moisture cheese which ripens rapidly is likely to develop a sharp flavor and may have some objectionable odor when the can is opened. A cheese that ripens normally will have no appreciable odor.

Dealers should be impressed with the fact that cheese, even in sealed cans, is a perishable food product which must be protected from extremes of temperature. Cheese heated above the melting point of the fat will become oily.

Certain types of cheese, as for instance, Swiss or Emmenthaler, cannot be ripened in cans, but it may be desirable to cut ripened cheese into prints and seal in cans or glass jars. Since the print can not be cut to fit the container without waste there will necessarily be considerably space around the print. Ripened cheese has passed the most active stage of gas formation and can not be depended upon to dilute the oxygen of the air enough to completely prevent mold growth. Under these conditions it is necessary to displace the air around the print with an inert gas such as carbon dioxide or nitrogen. Carbon dioxide is not, strictly speaking, an inert gas, but because it is heavier than air it is easier to handle than the lighter and more inert nitrogen. Moverover, it is comparatively inexpensive and is easily obtained in convenient cylinders. The air may be displaced by flowing a rapid stream of carbon dioxide into the container holding the cheese. The can should be sealed at once. This does not always displace the air completely and it is better to use some device by which the air can be exhausted from the container and replaced with gas. Automatic sealers for doing this are available, but in operating on a small scale improvised apparatus can be used.

Square or rectangular prints or slices of cheese in a round container leave so much space into which gas can be compressed before sufficient pressure is developed to swell the can that a valve may not be necessary unless the cheese is to be held for some time.

Ripened cheese in which the gas formation is slow could probably be marketed in this way with little danger of swelling. We have held sliced Swiss cheese in glass jars for 8 months without the development of undue pressure but eventually the pressure will become sufficient to bulge the can.

SUMMARY

A method of canning cheddar cheese direct from the press is described. The essential thing in this method is a valve which permits the escape of the gases formed in the ripening of the cheese without allowing the ingress of air.

The cheese ripens normally without loss of moisture, formation of rind or growth of mold.

The increased cost of cheese in this package is largely offset by the elimination of loss through evaporation, the necessity for paraffining and the care of the cheese in the curing room.

TESTS OF AN ICE REFRIGERATED COOLING UNIT UNDER CONTROLLED CONDITIONS*

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The initial investment and overhead costs appear to be disproportionately high for mechanically refrigerated milk cooling units for the small dairyman. The ice industry may have a possible market for ice on the farm and there is need for further data on ice cooling, so a series of tests were run on an ice refrigerator.[1]

The unit as shown in figure 1 consisted of an insulated cabinet including

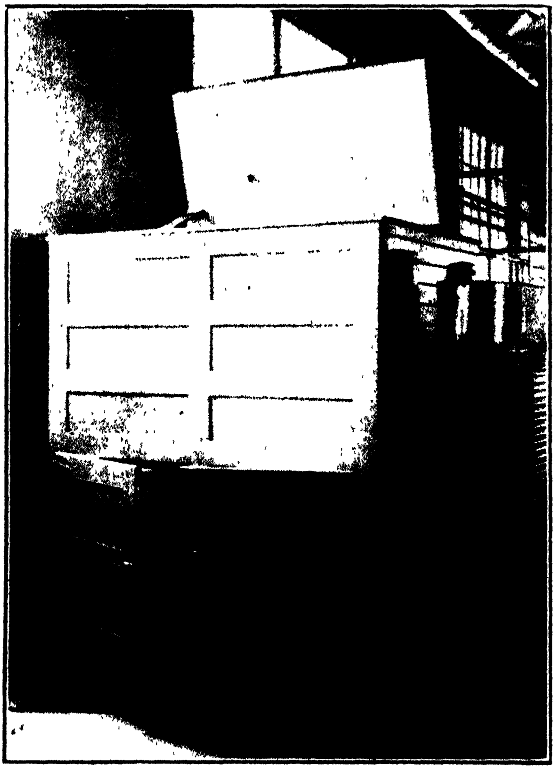

FIG. 1. ICE REFRIGERATED MILK COOLING UNIT.

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[1] The ice refrigerator used in these studies was built by the Horst Company, of Alameda, California.

an ice water tank of 1,180 pounds' capacity and a storage compartment for 6–10-gallon milk cans. The lower section of a two-way surface cooler 24 inches in width was cooled by ice water circulated by a 5 g.p.m. direct connected centrifugal pump, both being mounted on one end of the cabinet. A wire basket holding 270 pounds of ice was suspended at the top of the ice water tank.

The operating characteristics of equipment may be determined by two techniques, viz: (1) Comprehensive tests on one or more units to permit analyzing the influence of various controlling factors, and (2) Surveys of general data on a large number of units in the field to predict the range within which operation under generalized conditions will probably occur. The first method is required for tests of preliminary designs, or when there are not enough units included under each typical set of conditions to warrant conclusions regarding the effect of changes in operation. It has the great advantage of giving results which may be duplicated. The second method may be used where the human factor is predominant, where the laboratory cannot represent field operation and where a sufficient number are available for observation. Incomplete observations on a few units are usually valueless for interpretation by others because of uncertainty as to whether controlling factors are duplicated. Confirmation of laboratory tests by field observations is desirable.

More usable information on the initial design of ice refrigerated cooling unit could be secured in a limited time by the first method than by the second. This required operation in a room of constant adjustable temperature, and a routine method of loading. It was recognized that these conditions may not duplicate field operation, but they were essential if the inter-related effects of ice content and room temperature were to be determined. The pertinent information to be secured was: temperature obtainable, capacity, and ice consumption per gallon of milk. In order to analyze the box performance tests were divided into A—No load tests at 100°, 80°, and 60° room temperature to determine the box characteristics, and B—Load tests in an 80° room with loads of 20, 40 and 60 gallons per day.

In the no-load tests, the basket was filled with ice in pieces about 6″ square or less. To weigh the ice, the basket was suspended from a steelyard raised by a block and tackle. Weights were noted at 6-hour intervals until less than 60 pounds remained. Room temperature was recorded by a 24-hour thermometer on each side of the room. Box temperatures were recorded by a 24-hour thermometer on the wall near the right door, with its bulb about 6″ below the warm air duct, and a 7-day thermometer on the back wall about 6″ above the floor. The ice tank temperature was recorded by a 12-hour thermometer with a long spud inserted through the cover and through a tube in the ice basket to a point about 4″ below the bottom of the basket. The overflow tank temperature was noted by a hand thermometer each time the ice was weighed.

In the load tests, water was used to simulate milk. Since its quantity was determined by weighing, the only correction required was for specific heat. (The cooler would function slightly differently with water than with milk because of a different heat transfer coefficient. The error is not serious, however). The "milk" was warmed in a tank above the cooler reservoir, to which it was admitted by a float valve. Steady flow was thus secured for all runs. The rate of flow was adjusted to about 40 gallons per hour. The tap water for cooling the upper section was measured with a calibrated water meter, and maintained uniform by a mercury manometer indicator across the cooler connections. From thermometers inserted in the cooler connections and placed in the cooler troughs, temperatures were taken after the first minute and at three minute intervals during cooling. The cooled "milk" was caught in 10-gallon cans on a platform scale under the cooler. The "night's milking" was placed in the box and its temperature was taken when removed in the morning. The ice weight was taken before and after cooling and the elapsed time noted in order to correct for normal box maintaining meltage during the cooling period.

A typical log sheet for a load run is shown in figure 2. The breaks in the ice content curve indicate when milk was being cooled. It will be noted that the box temperature dropped at first following re-icing, and gradually

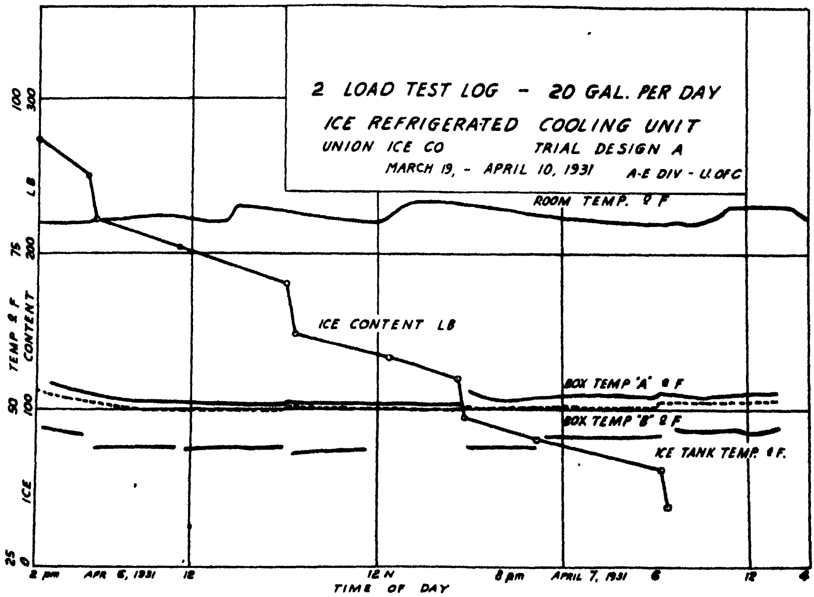


Fig. 2. Typical Load Test Log.

rose as the ice content decreased. The temperature of the ice tank at the observation point is considerably above 32°, indicating poor circulation.

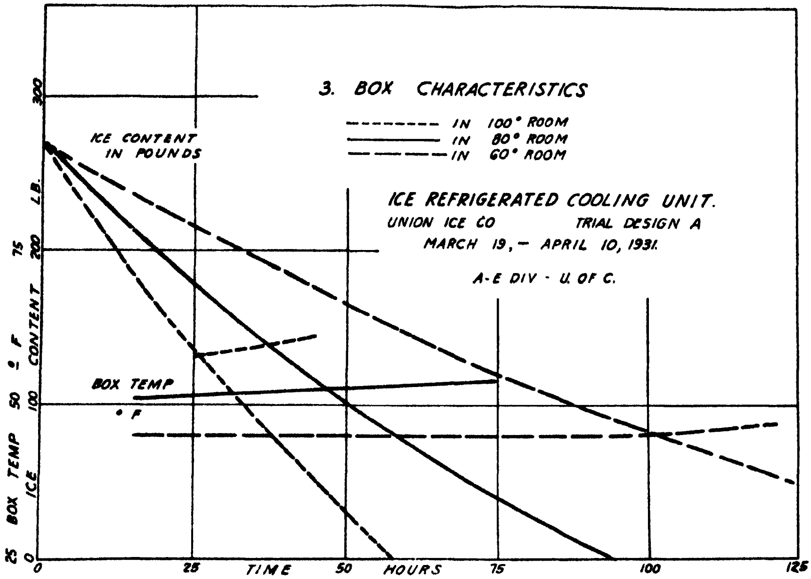


FIG. 3. BOX CHARACTERISTICS.

The no-load box characteristics are shown for different room temperature on figure 2. As the rate of meltage is proportional to ice surface exposed, the ice content curves might logically be logarithmic functions. Trial plotting on semi-logarithmic paper showed that straight lines obtained with the addition of appropriate constants to the ice weight, from which the following was derived:

$$T_2 - T_1 = k \log_{10} \frac{(W_1 + b)}{(W_2 + b)} \tag{1}$$

for the time in hours, $(T_2 - T_1)$ required for the ice weight to be reduced from W_1 pounds to W_2 pounds. Constants for this equation are given in Table 1.

TABLE 1
Constants for no load ice meltage
Equation (1)

ROOM TEMP.	k	b
100	203	280
80	287	240
60	450	200

The box temperature depends upon the ice content and the room temperature. Taking 50 pounds as the minimum ice content before re-icing, it was found possible to express the box temperature as a function of the room temperature, as follows:

$$T_b = (T_r - 32)^{0.77} + 32 \text{ in which } \frac{T_b = \text{box temp. °F.}}{T_r = \text{room temp. °F.}}$$

From the rate of heat loss of the box, and the difference in temperature between box and room, a "heat transfer constant" giving the B.t.u. per day per degree temperature difference between box and room is derived. Heat is derived from melting of ice, rise in temperature of melted ice from 32° to ice tank temperature, and rise in temperature of water in ice tank. The average heat transfer coefficient of 452 checks fairly well with the value of 475 derived from box dimensions and insulation.

A typical load data sheet is shown condensed in table 2 a, b, c.

TABLE 2a
Ice weights

TIME	ICE WT. NET LB.
6: 30 a. m.	175.5
7: 27 a. m.	130.5

TABLE 2b
Cooled milk data

CAN NO	NET WT. LB.	TEMP. AFTER COOLING °F.
2a	81.1	49
2b	81.1	46.5

TABLE 2c
Cooling data

CAN NO.	TIME	MILK TEMP. °F.		ICE WATER TEMP. °F.		TAP WATER TEMP. °F.		TAP WATER METER CU. FT.
		On	Off	On	Off	On	Off	
2a	6: 47							80.6
	: 48	88	50	42	46	72	74	
	: 51	88.5	48	40	45	72	73	
	: 54	89	46	40	45	71	73	
	: 57	89	46	39	44	71	73	
	7: 00	89	45	39	44	70	73	
	: 02							90.7
2b	7: 07							91.6
	: 08	88.8	45.5	39.3	44	71	73	
	: 11	89	44.5	38	44	71.5	73.5	
	: 14	89.2	44.2	38	43.5	71	73	
	: 17	89.6	44.8	38	43.5	70.3	73	
	: 20	90	44.5	38	43	70	73	
	: 22							102.2

The average operating conditions of the cooler are shown in figure 4. The intermediate milk temperature 74.7° F. and the mean temperature dif-

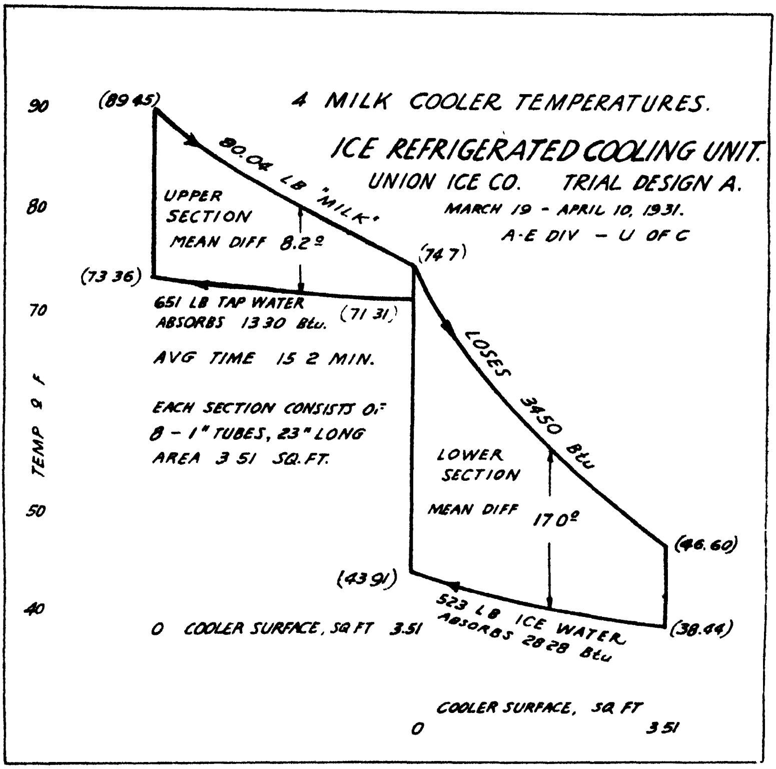


FIG. 4. MILK COOLER TEMPERATURES.

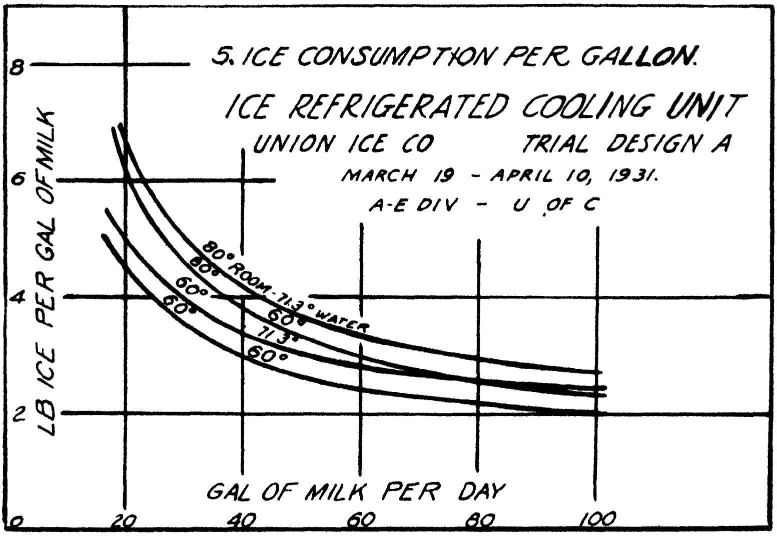
ferences were derived by calculation. The ice meltage during cooling per 10-gallon can is shown in Table 3.

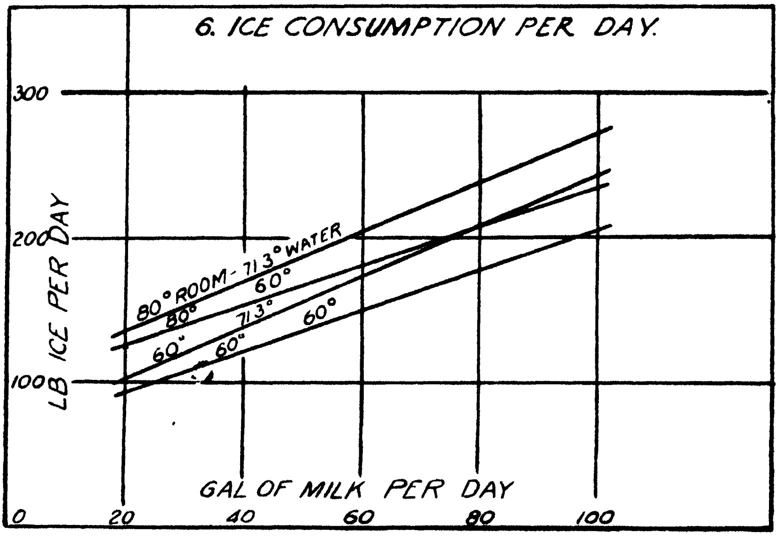
TABLE 3

Ice meltage for cooling alone

GAL. PER DAY	ICE MELTAGE LB. PER 10 GAL.
20	25.2
40	22.0
60	20.4

At first glance there was apparently no logical basis for a relation between the gallons per day and the ice meltage per gallon. However, when the heat absorbed by the ice-water in the lower cooler section has compared with the heat absorbed by ice meltage, it was found that an average of 6 pounds of ice was melted in excess of the cooler requirement in each cooling period, whether 10, 20 or 30 gallons was cooled. The temperature of the ice-water tank was reduced by an amount approximately equivalent to this heat removal, indicating that the increased circulation of the pump and spray during cooling caused a more rapid melting of the ice. Although this excess meltage did cause a slightly lower box temperature, it did not reduce the amount of ice consumed in box maintenance, so it was charged against the cooling operation. On this basis, the ice meltage for cooling alone for capacities of 20 to 100 gallons of milk per day were derived. Using rates of flow, initial temperatures of milk and ice-water,





FIGS. 5, 6. TOTAL ICE CONSUMPTION.

and heat transfer coefficient similar to those shown in figure 4, the ice meltages for operation with 60° F. tap water were obtained.

The ice meltage for box maintenance alone, from equation 1, and for milk cooling alone as derived in the above paragraph were added together to give the total ice consumption per gallon of milk, and per day, as shown in figure 5 and figure 6. These may be represented rather closely as follows:

$$I_g = c + \frac{m}{G} \tag{3}$$

$$I_d = c \cdot G + m \tag{4}$$

when I_g = ice meltage, lb./gal.
I_d = ice meltage, lb./day
G = gallons per day
c = constant for cooling alone
m = constant for box maintenance and cooler operation overhead.

TABLE 4

Constants for total ice meltage equations (3), (4)

ROOM TEMP. ° F.	TAP WATER TEMP. ° F.	c LB. /GAL.	m LB. /DAY
80	71.3	1.71	101
	60	1.37	98
60	71.3	1.75	67
	60	1.41	64

Although the ice consumption per gallon, as shown by figure 5, is excessive for small loads because of the meltage for box maintenance, it drops rapidly with increasing loads. The economic place of this equipment obviously depends upon whether the higher operating cost for ice plus lower fixed cost for the ice refrigerated unit is lower than the lower operating cost for power plus the higher fixed cost for the mechanically refrigerated unit.

CONCLUSIONS

1. Testing the ice refrigerated cooling unit under controlled conditions gave a fairly complete analysis of its operating characteristics in a short testing period.

2. With the box well iced, milk can be cooled and stored at 50° F., at an ambient temperature of 80° F. Redesign of the box might reduce this slightly. Daily peaks of 100° F. or higher will not cause material fluctuations in box temperatures because of the buffer effect of the ice-water tank.

3. The maximum milk cooling capacity of the box at high temperature is limited by its ice capacity. At 80° ambient temperature, 80 gallons per day will require daily icing. Redesign can increase this to 230 gallons per day with daily icing. However, the storage capacity is 70 gallons, which

would permit handling about 120 gallons per day when one milking is stored. This would allow icing on alternate days.

4. The ice consumption per gallon of milk at an average ambient temperature of 60° F. and a tap water temperature of 71.3° F. is 5 pounds per gallon for 20 gallons per day and 2.3 pounds per gallon for 80 gallons per day. The box might be redesigned to reduce the ice consumption at lower capacities, which would require reducing the storage space and maximum capacity.

5. The ice refrigerated cooling unit may offer a solution to the cooling problem of the small dairymen who can secure ice at $6.00 to $9.00 per ton as compared with electric power at 2 to 3 cents per kw.-hr.

ACKNOWLEDGMENT

The author wishes to express his appreciation of the assistance given in performing the tests by A. T. Peterson, W. H. Redit and P. H. Watson.

DEVELOPMENT OF THE ESCHERICHIA–AEROBACTER GROUP OF BACTERIA IN BUTTER

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In the studies that have been carried out at the Iowa Agricultural Experiment Station on butter showing various flavor defects, organisms belonging to the Escherichia-Aerobacter group have been frequently isolated. Occasionally, these organisms made up a considerable percentage of the total flora. Although the odors and flavors of the butter examined did not resemble the odors and flavors of milk in which Escherichia or Aerobacter species had grown, it seemed desirable to investigate the relationship of this group to butter from the standpoints of (1) the growth in butter under favorable temperature conditions and (2) the influence on the odor and flavor of butter. The studies involved only temperatures and holding periods that represent essentially the severe conditions to which butter may be exposed in retail establishments and homes since there is no evidence suggesting significant growth of organisms in butter held in commercial storage rooms.

METHODS

In the making of the butter the following procedure was used. Sweet cream of a good quality was pasteurized at 82.2° C. for fifteen minutes by placing the cream, in a metal container, in a water bath, raising the temperature of the water as rapidly as possible while the cream was being stirred, and then holding at the desired temperature. The cream was cooled in a water bath, distributed to sterile glass jars of approximately one gallon capacity, putting about 1500 ml. in each, and then held over night at about 7° C. to firm up the fat. The cream with which organisms were to be used was inoculated, just previous to churning, with from three to four ml. of a twenty-four to forty-eight hour milk culture of an identified organism that had been isolated from a dairy product. The churning was carried out in a shaking machine and usually required from twenty to twenty-five minutes. After churning, the buttermilk was poured off, the butter washed twice with sterile distilled water and then worked in a sterile enamel dish with a butter paddle. Each churning of butter was divided into two lots, one of which was salted while the other was left unsalted. Each lot was divided into two portions and wrapped in sterile parchment, one portion being held at about 7° C. while the other was held

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at about 18° C.; the variations from 7° and 18° C. were usually not over 1° C. and never over 2° C.

An attempt was made to incorporate about 2 per cent of salt into the salted lots but uniform incorporation was difficult with the salting procedure used; analyses showed that the salt content varied from 1.5 to 2.5 per cent.

The butter was plated as soon as it was made and after two, five and ten days, using eosin-methylene-blue agar plates in duplicate and incubating for two days at 37° C.; preliminary trials indicated that this temperature was very satisfactory for all the test organisms used. The medium employed made it possible to distinguish organisms of the Escherichia-Aerobacter group from other types.

The microscopic counts were made with the procedure used by Hammer and Nelson (1).

EXPERIMENTAL

Escherichia-Aerobacter Species Isolated from Off Flavored Butter

In order to secure information on the Escherichia-Aerobacter species present in butter, twenty-five cultures belonging to the general group were selected from the cultures isolated in the study of seventeen samples of off flavored butter and identified; a number of the samples of butter showed surface taint while the remainder showed various other objectionable odor and flavor conditions. Fifteen (60 per cent) of the cultures were *A. aerogenes,* four (16 per cent) were *A. cloacae,* three (12 per cent) were *A. oxytocum,* and three (12 per cent) belonged to the intermediate genus Citrobacter (2). None of the organisms studied belonged to the genus Escherichia.

Changes in the Numbers of Organisms of the Escherichia-Aerobacter Group in Butter

At about 7° C.—The counts secured on butter made from cream containing organisms of the Escherichia-Aerobacter group and held at about 7° C. are given in table 1. On the fresh unsalted butter the counts for the seven lots containing Escherichia species ranged from 110,000 to 2,800,000 per ml., with one sample exceeding 500,000 per ml., while comparable counts for the eight lots containing Aerobacter species ranged from 88,000 to 1,100,000 per ml., with three samples exceeding 500,000 per ml.

The salted butter containing Escherichia species gave counts from 21,000 to 130,000 per ml. after two days, from 7,500 to 57,000 after five days and from 6,500 to 60,000 after ten days; on the unsalted butter the counts ranged from 130,000 to 11,000,000 per ml. after two days, from 85,000 to 20,000,000 after five days and from 85,000 to 5,200,000 after ten days.

TABLE 1

Organisms of the Escherichia-Aerobacter group, according to eosin-methylene-blue agar plate counts, in butter held at about 7° C.

SPECIES INOCULATED INTO CREAM	CULTURE DESIGNATION	NUMBER PER ML. IN						
		FRESH UNSALTED BUTTER	SALTED BUTTER AFTER			UNSALTED BUTTER AFTER		
			2 days	5 days	10 days	2 days	5 days	10 days
SPECIES BELONGING TO GENUS ESCHERICHIA								
E. coli	C8	2,800,000				11,000,000	20,000,000	4,700,000
E. communior	M2	300,000				830,000	360,000	660,000
E. coli	C1	300,000	130,000	7,500	7,900	530,000	85,000	5,200,000
E. coli	C8	110,000	29,000	17,000	6,500	130,000	97,000	150,000
E. formica	M14	260,000	21,000	43,000	16,000	160,000	99,000	85,000
E. communior	M2	490,000	52,000	50,000	60,000	300,000	830,000	2,500,000
E. paragrünthali	BM4	160,000	61,000	57,000	40,000	210,000	250,000	300,000
SPECIES BELONGING TO GENUS AEROBACTER								
A. aerogenes	C16	1,100,000					71,000,000	61,000,000
A. oxytocum	C23	980,000				16,000,000	76,000,000	> 30,000,000
A. cloacae	C11	420,000				740,000	2,600,000	4,900,000
A. aerogenes	C16	88,000	9,000	69,000	>500,000	230,000	75,000	> 1,000,000
A. oxytocum	C23	220,000	18,000	5,000	120,000	190,000	330,000	> 1,000,000
A. cloacae	C11	280,000	110,000	330,000	180,000	310,000	3,300,000	17,000,000
A. cloacae	H1	1,000,000		420,000	800,000	1,000,000	6,500,000	18,000,000
A. aerogenes	H3	140,000	20,000	510,000	1,800,000	500,000	4,100,000	9,000,000

The salted butter containing Aerobacter species showed counts of from 9,000 to 110,000 per ml. after two days, from 5,000 to 510,000 after five days and from 120,000 to 1,800,000 after ten days; on the unsalted butter the counts varied from 190,000 to 16,000,000 per ml. after two days, from 75,000 to 76,000,000 after five days and from more than 1,000,000 to 61,000,000 after ten days.

In general, at about 7° C., salt had a definite restraining action on the organisms of the Escherichia-Aerobacter group; in the salted butter the Escherichia species did not grow in the ten day period and commonly there was a pronounced decrease in numbers, while with the Aerobacter species there was usually a decrease and then an increase in numbers, the increase in some instances resulting in counts higher than the originals. In the unsalted butter some of the Escherichia and all of the Aerobacter species studied grew, with the latter showing more growth than the former.

At about 18° C.—The counts secured on butter made from cream containing organisms of the Escherichia-Aerobacter group and held at about 18° C. are given in table 2. As already indicated these lots of butter represent the same churnings as the butter held at about 7° C.

The salted butter containing Escherichia species gave counts from 1,200,000 to 1,900,000 per ml. after two days, from 1,100,000 to 17,000,000 after five days and from 1,200,000 to 12,000,000 after ten days; on the unsalted butter the counts varied from 4,900,000 to 30,000,000 per ml. after two days, from 11,000,000 to 38,000,000 after five days and from 20,000,000 to 35,000,000 after ten days.

The salted butter containing Aerobacter species showed counts from 1,900,000 to 22,000,000 after two days, from 3,000,000 to 50,000,000 after five days and from 6,800,000 to 28,000,000 after ten days; on the unsalted butter the counts ranged from 14,000,000 to 100,000,000 after two days, from 46,000,000 to 290,000,000 after five days and from 50,000,000 to 150,000,000 after ten days.

At about 18° C. salt had a restraining action on the organisms of the Escherichia-Aerobactor group but growth occurred with all the cultures studied in both the salted and the unsalted butter. The Aerobacter species grew more rapidly and reached higher numbers in the butter than the Escherichia species.

Influence of Organisms of the Escherichia-Aerobacter Group on the Odor and Flavor of Butter

Most of the butter made from cream to which organisms of the Escherichia-Aerobacter group had been added was examined after two, five and ten days for odor and flavor, and comparisons made with the controls. The results obtained are given in table 3. Due to the pasteurization at comparatively high exposures under conditions such that thorough agita-

TABLE 2

Organisms of the Escherichia-Aerobacter group, according to eosin-methylene-blue agar plate counts, in butter held at about 18° C.

SPECIES INOCULATED INTO CREAM	CULTURE DESIGNATION	NUMBER PER ML. IN						
		FRESH UNSALTED BUTTER	SALTED BUTTER AFTER			UNSALTED BUTTER AFTER		
			2 days	5 days	10 days	2 days	5 days	10 days
SPECIES BELONGING TO GENUS ESCHERICHIA								
E. coli	C8	2,800,000				17,000,000	23,000,000	33,000,000
E. communior	M2	300,000				30,000,000	38,000,000	35,000,000
E. coli	C1	300,000	1,200,000	1,500,000	1,200,000	25,000,000	21,000,000	20,000,000
E. coli	C8	110,000	1,200,000	2,300,000	1,200,000	18,000,000	19,000,000	23,000,000
E. formica	M14	260,000	1,200,000	1,100,000	8,300,000	16,000,000	11,000,000	22,000,000
E. communior	M2	490,000	1,900,000	17,000,000	12,000,000	30,000,000	28,000,000	29,000,000
E. paragrünthali	BM4	160,000		9,700,000	12,000,000	4,900,000	35,000,000	33,000,000
SPECIES BELONGING TO GENUS AEROBACTER								
A. aerogenes	C16	1,100,000				56,000,000	56,000,000	68,000,000
A. oxytocum	C23	980,000				54,000,000	70,000,000	80,000,000
A. cloacae	C11	420,000				14,000,000	290,000,000	110,000,000
A. aerogenes	C16	88,000	22,000,000	23,000,000	6,800,000	100,000,000	46,000,000	50,000,000
A. oxytocum	C23	220,000	2,100,000	3,000,000	11,000,000	16,000,000	100,000,000	130,000,000
A. cloacae	C11	280,000	1,900,000	26,000,000	18,000,000	32,000,000	170,000,000	110,000,000
A. cloacae	H1	1,000,000	22,000,000	48,000,000	28,000,000	70,000,000	190,000,000	150,000,000
A. aerogenes	H3	140,000	13,000,000	50,000,000	19,000,000	70,000,000	78,000,000	78,000,000

TABLE 3

Odors and flavors in butter containing organisms of the Escherichia-Aerobacter group

SPECIES	NO. CULTURES USED	ODOR AND FLAVOR AFTER HOLDING AT											
		ABOUT 7° C.						ABOUT 18° C.					
		SALTED BUTTER			UNSALTED BUTTER			SALTED BUTTER			UNSALTED BUTTER		
		2 days	5 days	10 days	2 days	5 days	10 days	2 days	5 days	10 days	2 days	5 days	10 days
E. coli	3	3–	3–	3–	3–	3–	3–	3–	3–	3–	3–	3–	3–
E. communior	2	2–	2–	2–	2–	2–	2–	2–	2–	2–	2–	2–	2–
E. formica	1	1–	1–	1–	1–	1–	1–	1–	1–	1–	1–	1–	1–
E. paragrünthali	1	1–	1–	1–	1–	1–	1–	1–	1–	1–	1–	1–	1–
A. aerogenes	3	3–	3–	3–	3–	3–	2– 1+	3–	3–	1+ 2++	1– 1+ 1++	1– 2++	3+++
A. oxytocum	2	2–	2–	2–	2–	2–	1– 1++	1+	1+	1+	1– 1++	1– 1++	2+++
A. cloacae	2	2–	2–	2–	2–	2–	1+ 1++	2–	2–	2++	1– 1+	1– 1++	2+++

– no off odor or flavor not present in check.
+ slight unclean odor and flavor.
++ pronounced unclean odor and flavor.
+++ very pronounced unclean odor and flavor.

tion was impossible, all the butter had a rather pronounced heated flavor. The unsalted control butter occasionally developed an off flavor, due presumably to the growth of organisms surviving the pasteurization, but this was readily distinguishable from the defects caused by the Escherichia-Aerobacter species.

At about 7° C.—The butter containing Escherichia species did not develop off flavors due to the organisms added to the cream, either when it was salted or when it was not. The butter containing Aerobacter species did not develop off flavors due to the organisms added to the cream when it was salted but, when unsalted, off flavors were present after ten days with one of three *A. aerogenes* cultures, with one of two *A. oxytocum* cultures and with the two *A. cloacae* cultures.

At about 18° C.—The butter containing Escherichia species did not develop off flavors due to the organisms added to the cream, either when salted or when unsalted. The butter containing Aerobacter species developed off flavors both when salted and when unsalted; when salted, an off flavor was regularly present after ten days, and with the one *A. oxytocum* culture after two days, while when unsalted an off flavor was regularly present after ten days and with two of three *A. aerogenes* cultures, with one of two *A. oxytocum* cultures and with one of two *A. cloacae* cultures after two days.

General character of the odor and flavor produced in butter by Aerobacter species.—The odor and flavor defect produced in butter by the Aerobacter species studied would probably be designated as an unclean odor and flavor by most butter judges. It very definitely resembled the condition produced in milk by these organisms.

The odors and flavors produced in milk by Escherichia species are, in general, definitely less objectionable than those produced by the Aerobacter species. With a considerable number of cultures belonging to the general Escherichia-Aerobacter group (including the genus Citrobacter), those belonging to the genus Aerobacter commonly could be picked out on the basis of the odor produced in milk. This suggests that even if Escherichia species did produce odor and flavor changes in butter they would be less objectionable than those produced by Aerobacter species. No differences could be detected between the odors produced in milk by the various Aerobacter species studied.

Retention in Butter of Organisms Belonging to the Escherichia-Aerobacter Group

In order to study the retention in butter of organisms belonging to the Escherichia-Aerobacter group, a count of these organisms was made on a number of lots of cream just before churning. The results obtained, together with the counts secured on the fresh, unsalted butter and the percentages of the organisms retained in the butter, are presented in table 4.

TABLE 4

Organisms of the Escherichia-Aerobacter group in cream and in butter churned from it

Counts made with eosin-methylene-blue agar plates

SPECIES INOCULATED	SERIES	CULTURE DESIGNATION	NUMBER PER ML. IN		PER CENT OF ORGANISMS PER ML. OF CREAM RETAINED PER ML. OF BUTTER
			Inoculated cream	Fresh, unsalted butter	
A. aerogenes	A	C16	30,000,000	1,100,000	3.7
A. oxytocum	B	C23	23,000,000	980,000	4.3
E. coli		C8	14,000,000	2,800,000	20.0
A. cloacae	C	C11	7,600,000	420,000	5.5
E. communior		M2	5,300,000	300,000	5.7
A. aerogenes	D	C16	1,300,000	88,000	6.8
E. coli		C8	2,100,000	110,000	5.2
A. oxytocum	E	C23	2,100,000	220,000	10.5
E. formica		M14	3,400,000	260,000	7.6
A. cloacae	F	C11	9,600,000	280,000	2.9
E. communior		M2	2,900,000	490,000	16.9
A. cloacae	G	H1	14,000,000	1,000,000	7.1
A. aerogenes		H3	2,100,000	140,000	6.7
E. paragrunthali		BM4	1,800,000	160,000	8.9

The data show that from 2.9 to 20.0 per cent of the organisms present per ml. of the cream were retained per ml. of fresh, unsalted butter, with the percentage less than 10 in eleven of the fourteen comparisons. There was no significant difference between the retention of organism of the Escherichia and of the Aerobacter species and no correlation between the retention of the organisms and the number present in the cream.

Grimes (3) found that, with butter churned from pasteurized sweet cream, from 5 to 30 per cent (generally between 20 and 30 per cent) of the bacteria per ml. of cream were retained per ml. of butter.

Ratio of Microscopic Counts to the Number of Organisms of the Escherichia-Aerobacter Group, According to Eosin-Methylene-Blue Plate Counts, in Butter Made from Inoculated Cream

Microscopic counts were compared with the number of organisms of the Escherichia-Aerobacter group, as determined by eosin-methylene-blue agar plate, on twelve of the samples of butter made from cream to which an Escherichia or an Aerobacter species had been inoculated; the ages of the samples when examined varied. The results obtained are given in table 5. The data show that the ratios varied from 1.0 to 1 to 18.4 to 1, with the ratio being less than 3 to 1 in seven of the twelve comparisons. There was no

TABLE 5

Ratio of microscopic count to the number of organisms of the Escherichia-Aerobacter group, according to eosin-methylene-blue plate counts, in butter made from inoculated cream

SAMPLE NUMBER	BACTERIA PER ML.		RATIO OF MICROSCOPIC TO PLATE COUNT
	Microscopic count	Eosin-methylene-blue plate count	
1	2,700,000	420,000	6.4: 1
2	590,000	300,000	2.0: 1
3	5,200,000	740,000	7.0: 1
4	1,800,000	830,000	2.2: 1
5	210,000	130,000	1.6: 1
6	1,500,000	530,000	2.8: 1
7	2,200,000	360,000	6.1: 1
8	2,900,000	1,200,000	2.4: 1
9	34,000,000	25,000,000	1.4: 1
10	35,000,000	1,900,000	18.4: 1
11	31,000,000	30,000,000	1.0: 1
12	31,000,000	1,900,000	16.3: 1

correlation between the ratio and the number of organisms in the butter; the four samples having the largest numbers of bacteria gave the two highest and also the two lowest ratios.

The microscopic preparations showed the organisms in the butter to be mainly isolated or in pairs, and the eosin-methylene-blue plates indicated that nearly all of them belonged to the Escherichia-Aerobacter group. Under these conditions the large variations in the ratios are rather surprising. Some of the organisms may have been dead and still definitely stainable; the plate counts reported in tables 1 and 2 indicate some destruction of organisms in comparatively short periods. Another factor that may be of considerable importance is the failure of the higher dilution plates to show as many organisms as would be expected from the lower dilution plates; this tendency has been noted at the Iowa Agricultural Experiment Station in the study of various butter problems.

DISCUSSION OF RESULTS

The data reported show that both comparatively low temperatures and salt have striking influences in restraining the growth of organisms of the Escherichia-Aerobacter group in butter. The cumulative effect of unfavorable conditions is evident from the fact that growth may occur in salted butter at about 18° C. and in unsalted butter at about 7° C. when it will not occur in salted butter at about 7° C. In general, the results obtained on the organisms of the Escherichia-Aerobacter group in butter are in agreement with the results that have been reported for a varied flora.

The more rapid growth of the Aerobacter than of the Escherichia species in butter may be the explanation for the abundance of Aerobacter species among the organisms of the Escherichia-Aerobacter group isolated from off flavored butter. This more rapid growth, together with the development of an objectionable odor and flavor by the Aerobacter species, makes them of greater importance than the Escherichia species from the standpoint of butter deterioration. Even with the Aerobacter species, however, there was commonly considerable growth in butter without any evident influence on the odor and flavor.

CONCLUSIONS

1. Twenty-five cultures, belonging to the Escherichia-Aerobacter group, isolated from seventeen samples of off flavored butter included fifteen cultures of *A. aerogenes*, four of *A. cloacae*, three of *A. oxytocum* and three belonging to the intermediate genus Citrobacter.
2. In ten days at about 7° C., Escherichia species did not grow in salted butter but in unsalted butter some of them did while Aerobacter species sometimes grew in the salted butter and regularly grew in the unsalted.
3. In ten days at about 18° C., both Escherichia and Aerobacter species grew in salted as well as unsalted butter, but the Aerobacter species grew more rapidly and reached higher numbers than the Escherichia species.
4. In ten days at about 7° C., Escherichia species did not develop off odors and flavors in butter, either when salted or unsalted; Aerobacter species did not develop off odors and flavors in salted butter but sometimes did in unsalted butter.
5. In ten days at about 18° C., Escherichia species did not develop off odors and flavors in butter either when salted or unsalted; Aerobacter species regularly developed off odors and flavors in either salted or unsalted butter.
6. The odor and flavor produced in butter by Aerobacter species definitely resembled the condition produced in milk by these organisms.
7. From 2.9 to 20.0 per cent of the organism of the Escherichia-Aerobacter group present per ml. of cream were retained per ml. of fresh, unsalted butter.
8. The ratio of microscopic counts to the numbers of organisms of the Escherichia-Aerobacter group according to the eosin-methylene-blue counts in butter made from inoculated cream varied from 1.0 to 1 to 18.4 to 1.

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THE EFFECT OF CORN OIL ON MILK AND BUTTER FAT PRODUCTION AND ON THE COMPOSITION OF BUTTER FAT IN THE DAIRY COW

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In a previous report (1), it was shown that the feeding of menhaden oil to a dairy cow resulted in a marked decline in the milk production and an even more decided reduction in butter fat. The suggestion was made that this remarkable effect might be due to one of three factors,—the vitamin D content of the oil, the high fat diet, or to the characteristic highly unsaturated fatty acids of the fish oil. Naturally there might be some other unknown factor or combination of factors which produced these results. That the first of these is not the important factor has been shown by Bethke and Krauss (2), of the Ohio Experiment Station, who fed dairy cows on rations to which viosterol (irradiated ergosterol) was added without effect on the milk and butter fat yield.

The present investigation was carried out to determine the effect of adding corn oil in relatively large amounts to the diet of a dairy cow on the secretion of milk and butter fat and on the composition of the latter. In contrast to the results from menhaden oil the corn oil feeding showed very little effect. The chemical composition of the butter fat, however, was almost as much altered by the corn oil as by the fish oil. From these results and those of Bethke and Krauss it would appear that the cause of lowered milk and butter yield lies neither in the ingestion of large amounts of vitamin D or in a high fat diet, but is more probably due to some specific effect of the menhaden oil, either its characteristic unsaturated fatty acids or some other unknown factor in the oil.

EXPERIMENTAL PART

A Holstein cow, weighing 1310 pounds, in her ninth month of lactation was fed 5 pounds a day of a basic ration consisting of:

Ground oats	2 parts
Distiller's grains	2 "
Soy-bean meal	1 "
Linseed meal	1 "

and 2.25 pounds of starch for a control period of 8 days. For the following 16 days one pound of corn oil was substituted for the starch. The con-

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trol diet was then resumed. During each of these periods silage was given *ad libitum* along with a liberal amount of alfalfa hay.

The milk from two-day samples was tested for butter fat, and the butter fat was recovered and analyzed.

TABLE I

The effect of corn oil on the milk and butter fat yield

SAMPLE—DAY	MILK YIELD LBS. DAILY	PER CENT BUTTER FAT	LBS. BUTTER FAT LBS. DAILY
	Control feeding		
2	12.6	4.2	0.53
4	14.0	4.2	0.59
6	12.1	3.9	0.47
8	13.4	4.1	0.56
	Corn oil feeding begins		
10	13.4	3.9	0.52
12	12.3	3.8	0.47
14	12.2	3.9	0.48
16	11.8	3.6	0.43
18	11.3	4.0	0.46
20	12.8	3.9	0.50
22	14.2	3.5	0.49
24	12.7	3.4	0.43
	Corn oil feeding ends		
26	13.1	3.4	0.45
28	13.9	3.0	0.42
30	13.6	3.4	0.46

Specimens of the butter fat were analyzed by the usual methods with the results given in Table II.

DISCUSSION OF RESULTS

The data in Table I show little if any effect on milk yield, the per cent butter fat in the milk or in total butter fat production. A reasonably high fat diet, therefore, of itself has little quantitative influence on lactation. On the other hand, analyses of the butter (Table II), as in the previous report, reveal a decided change in the composition of the fat which is secreted by the mammary glands. A maximum increase of 30 per cent in the iodine number and a decrease in the volatile soluble acids to about one-half the normal value are changes worthy of note. This change of butter fat by diet must be kept in mind in the detection of adulteration. The secretion of butter fat which remains liquid at ordinary temperature is quite possible.

So far as explaining the effect of fish oil in altering the actual amount of milk and fat secretion, these results are significant only in ruling out one of the possible explanations suggested previously.

TABLE II

Analysis of butter fat

SAMPLE DAY	IODINE NUMBER	SAPONIFICATION NUMBER	REICHERT-MEISSL NO.
	Control period		
2	41.4	221.0	23.4
4	41.9	224.2	24.8
6	37.1	227.5	25.5
8	37.1	225.2	23.2
	Corn oil feeding begins		
10	42.1	225.5	22.1
12	51.3	216.5	19.4
14	50.6	216.6	19.1
16	52.1	214.5	17.4
18	51.0	213.0	19.9
20	52.9	214.2	17.3
22	54.2	213.8	16.7
24	55.8	210.9	14.0
	Corn oil feeding ends		
26	53.7	214.0	14.4
28	51.0	214.9	15.7
30	46.7	216.1	17.9
Corn oil	124.6	194.1	0.0

SUMMARY

(1) The ingestion of one pound per day of corn oil by a dairy cow showed no significant changes in milk or butter fat production.

(2) The corn oil decidedly changed the composition of the butter fat. The fatty acids were more unsaturated, of higher mean molecular weight, and the content of volatile soluble acids was reduced.

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THE FUNCTIONAL RELATION OF YEARLY FEED COST AND BUTTERFAT PRODUCTION IN DAIRY CATTLE

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The yearly butterfat production of dairy cows is regulated and influenced by a number of hereditary and physical factors. The ability of an animal to produce a large amount of butterfat in a year is inherited. Unless the physical factors involved in butterfat production are adequately provided maximum production cannot prevail.

Many of the influential physical factors have been studied by various workers. When correlating them with yearly production they have found varying degreees of relationship to exist. Of the ten factors studied by Pound and Ezekiel (1) six were found to show a creditable degree of correlation with yearly butterfat production. Seventy-five per cent of the variation in the average butterfat yield of the herds studied could be accounted for by the following six factors mentioned in order of their importance: quality of management; nutritive ratio; total nutrients; fall freshening; proportion of silage in the ration, and fat test. When their results were measured in terms of milk production, percentage fat test was the more important factor. A multiple correlation of .87 was found to exist between these factors and yearly fat production. Ezekiel, McNall and Morrison (2) found a multiple correlation of .71 between the nutritive ratio, total nutrients, and fat test and the average yearly milk production in certain Wisconsin herds.

That certain of these factors affected the annual feed cost of fat production was borne out by them. In the herds observed it was illustrated that by adding protein to the ration the cost was greater but the cash return above feed cost was increased. Data presented by Cramer (3) show that cows producing 400 pounds of fat in a year had a higher feed cost than cows producing only 100 pounds but that the return above cost of feed was much greater in the case of the higher producing cows.

The relationship between annual feed cost and butterfat production in dairy cows has been elucidated in a recent paper by McIntyre (4). He

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* The material included in this paper was collected by the author when matriculated in the College of Agriculture, University of Wisconsin.

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found a correlation of .5641 ± .007 between the two variables and presents equations showing the maintenance costs and the cost of producing 100 pounds of milk. He relates that 600 pound butterfat cows have a 66.7 per cent greater feed cost than 300 pound cows, but that the feed cost per pound of fat is less in the high producing group.

This paper records a description of the functional relation between yearly feed cost and butterfat production of dairy cows. Data are presented to show the relationship between production and the cost of producing a unit of butterfat at various production levels.

METHODS

The data used in determining the correlation between yearly feed cost and butterfat production of dairy cows were obtained from 10,000 individual Dairy Herd Improvement Association records. These records were made by cows generally distributed over the State of Wisconsin during 1926 to 1928 inclusive. Records were taken at random and factors other than those involved in this paper were not considered. Records of less than 8 months' duration were not included in these data.

The records were classified for feed cost and butterfat production on the usual form of correlation table. Feed cost was divided into $10.00 intervals while butterfat production was arranged in 50 pound intervals. The original data showing the number of records in each class interval together with the final frequency distribution are shown in Chart I. The correlation analysis and other calculation were performed as outlined by Holzinger (5) and Ezekiel (6).

RESULTS

The 10,000 records under study were arranged into a feed cost frequency distribution curve as shown in Figure 1. The class intervals were $10.00 and covered a range in yearly feed cost from $15.00 to $165.00. No feed costs were found to occur outside this range. The mean cost of the records studied were $59.36 ± .043 with a standard deviation of $16.43 ± .116.

Figure 2 represents the frequency distribution for yearly butterfat production. The records arranged themselves into 11 different production intervals of 50 pounds each which ranged from 125 to 675 pounds. The mean yearly butterfat production was 284.57 ± .524 pounds and the standard deviation was 77.50 ± .548 pounds.

Since no records of less than 8 months' duration were included the lower limit of occurrence was restricted to 125 pounds. Had all records been included the frequency curve would probably have exhibited approximately the same degree of variation below the mean as it did above. It was assumed that records shorter than 240 days did not give an accurate measure

CHART I

Data classified according to annual production and feed cost.

Yearly Feed Cost	Annual Production											
	125 174.9	175 224.9	225 274.9	275 324.9	325 374.9	375 424.9	425 474.9	475 524.9	525 574.9	575 624.9	625 674.9	
155–164.9											1	1
145–154.9												
135–144.9												
125–134.9					1		2	3	1	1	1	9
115–124.9					1	1	4	2	1		1	10
105–114.9		1	2	8	6	14	7	3	2			43
95–104.9		2	11	40	40	42	21	9	3	1		169
85– 94.9	1	4	36	72	115	73	46	26	8	5	1	387
75– 84.9	7	27	124	283	261	197	87	30	5	1	1	1,023
65– 74.9	12	150	415	531	440	184	53	17	4	1		1,807
55– 64.9	75	328	690	643	407	155	51	9	2			2,362
45– 54.9	155	536	680	546	267	84	17	3				2,288
35– 44.9	225	422	411	245	69	12	3	1				1,388
25– 34.9	153	138	109	35	6							441
15– 24.9	27	31	8	5	1							72
Frequency	655	1,639	2,486	2,408	1,616	762	291	103	26	9	5	10,000

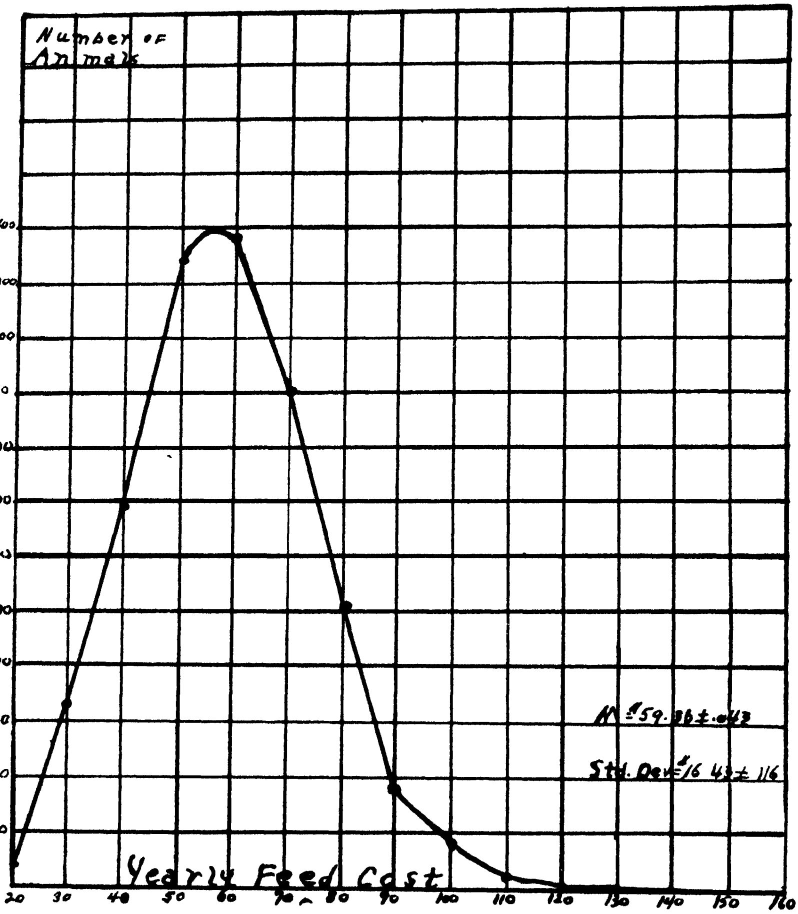

FIG. 1. FREQUENCY DISTRIBUTION OF YEARLY FEED COST.

of a cow's producing ability, therefore, they were excluded from this report.

The correlation coefficient between yearly feed cost and butterfat production was determined to be .5390 ± .0048. This coefficient is positive and significant regardless of the many factors existing which may cause variation in both feed cost and production. The correlation found in this analysis agrees very favorably with that obtained by McIntyre (4) in a similar study.

The coefficient of determination of .29 shows that within the data included in this investigation 29 per cent of the variance in feed cost could be accounted for by differences in production. That the correlation obtained is to a high degree linear is substantiated by a correlation ratio of .556 as compared with the correlation coefficient of .5390. The regression equation which makes it possible to determine the feed cost of a given pro-

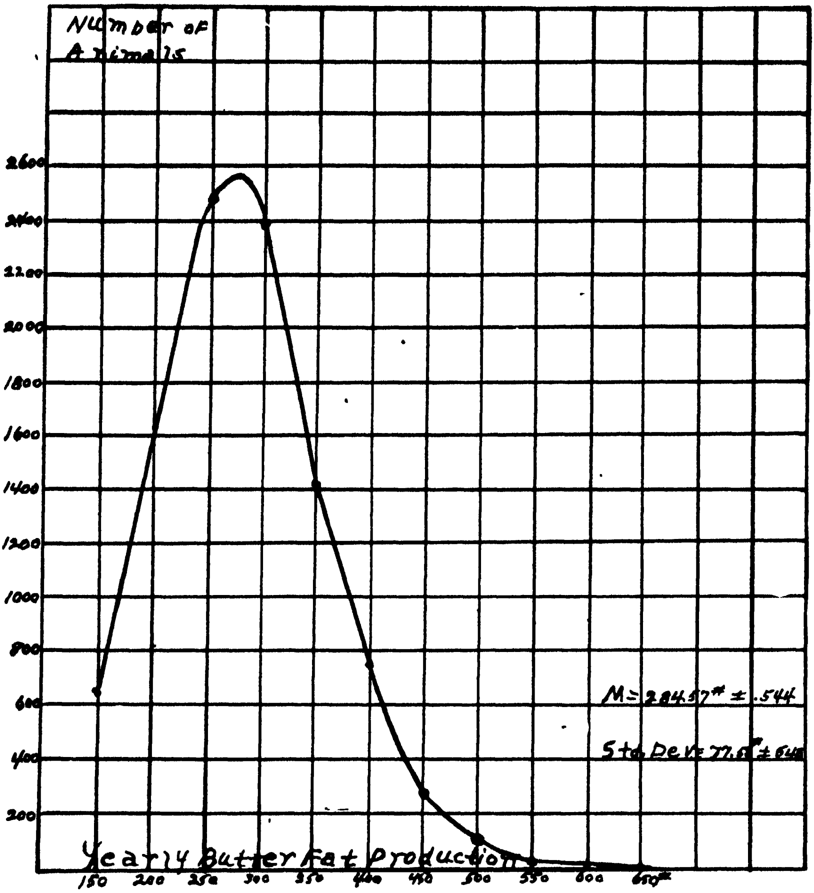

FIG. 2. FREQUENCY DISTRIBUTION OF YEARLY BUTTERFAT PRODUCTION.

duction is $y = .114x + 26.92$. In this equation y represents yearly feed cost and x annual butterfat production.

Figure 3 shows the regression curve for feed cost and production as determined by the regression formula. The independent variable x in each case was the midpoint of the various production intervals. The accuracy of this equation is confirmed by the closeness to which the average feed cost of each production group coincide with the points on the regression line. The crosses on the graph indicate the average feed cost for each interval. In the 600 and 650 pound groups the frequencies were too few to give conclusive results, which probably accounts for the divergence of the averages from the regression line.

This regression equation indicates that in these data there was an increase in feed of \$5.70 for each 50 pound increase in production, a fact which holds true regardless of the level of production. It further indicates

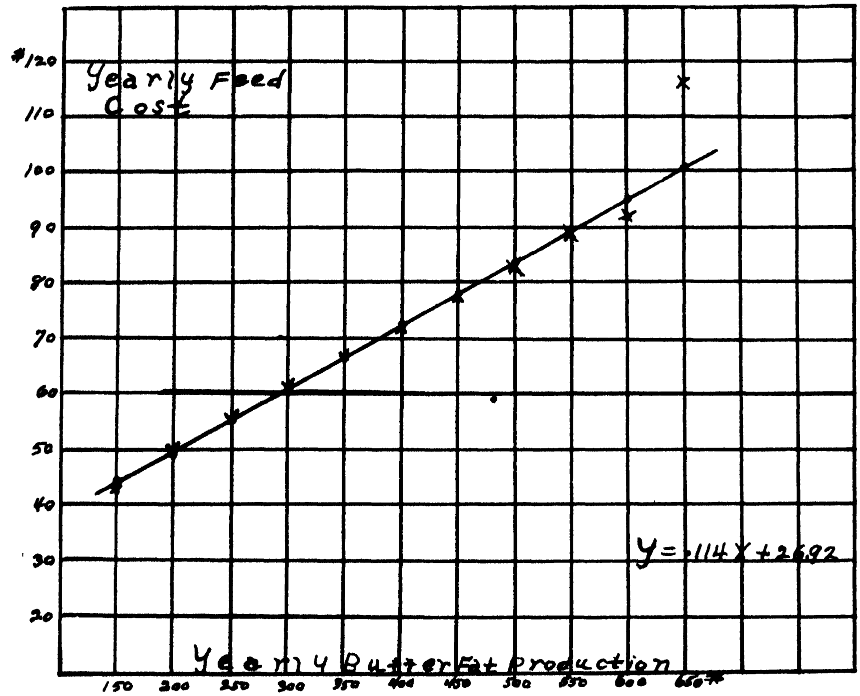

FIG. 3. RELATION OF YEARLY FEED COST TO BUTTERFAT PRODUCTION.

that if no butterfat was produced a feed cost of $26.92 would occur which represents the cost of maintenance. This maintenance cost is the same for both high and low producing cows.

The cost of producing one pound of butterfat for the various production levels is plotted in chart 4. This representation indicates that as production increases the cost of producing a pound of fat decreases rapidly until a yearly production of 500 pounds is reached, after which a less pronounced relationship operates. The cows producing 500 pounds of butterfat in a year produced a unit of fat at 24 per cent less cost than did cows producing 250 pounds.

CONCLUSIONS

The work herein reported constitutes a study of the functional relation between yearly feed cost and butterfat production of 10,000 individual Dairy Herd Improvement Association Records. A correlation of .5390 ± .0048 was found to exist between the two variables.

These data indicate that within the groups studied a unit increase in production was associated with a proportionate smaller increase in feed cost.

The data indicate that the cost of producing one pound of butterfat decreased as production increased.

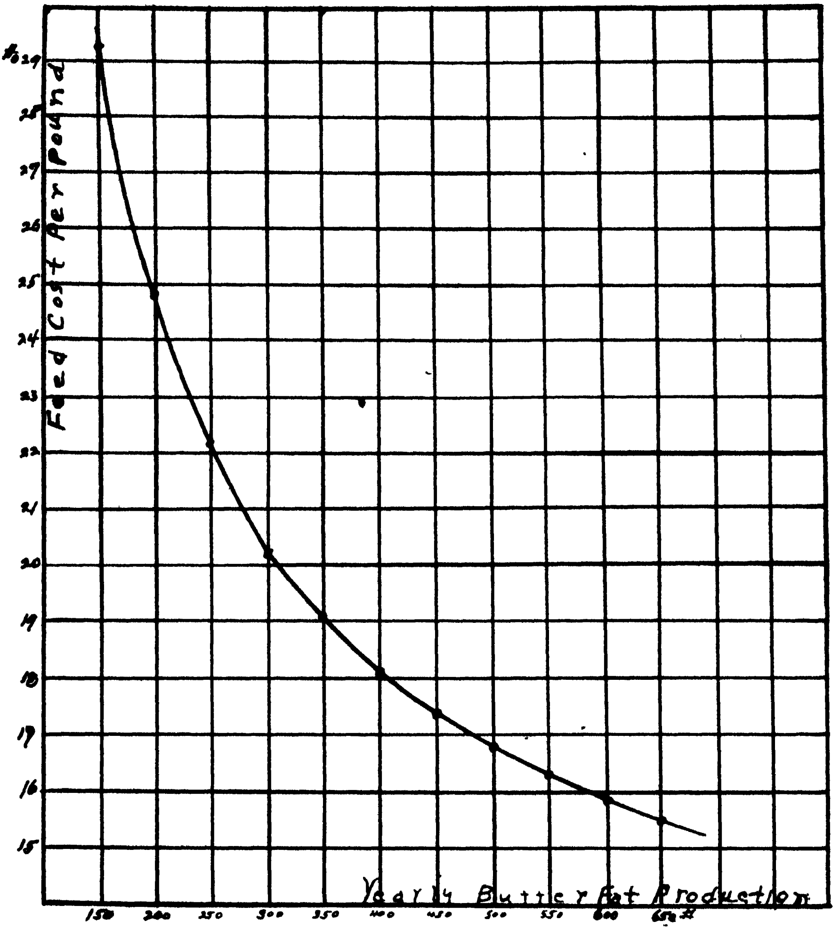

FIG. 4. RELATION OF YEARLY BUTTERFAT PRODUCTION TO COST OF PRODUCING ONE POUND OF BUTTERFAT.

The writer is indebted to Associate Professor I. W. Rupel, of the Department of Animal Husbandry, University of Wisconsin, for his able assistance in collecting and summarizing these data. Acknowledgment is due M. Wayne Miller, Poultryman, Western Washington Experiment Station, who assisted in interpreting the statistical results.

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ACTION OF AN AEROBIC SPORE-FORMING ORGANISM ON EVAPORATED MILK

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During the abnormally hot summer of 1930, a condensery shipping evaporated milk to various cities in the middle west, received reports of coagulation in the product. The condition varied from a very few small masses of soft curd in a can, which probably would not have been criticized if a more serious defect had not been present in other cans, to complete coagulation; with complete coagulation the curd was sometimes soft and in other cases quite firm.

Many cans of the spoiled milk were studied bacteriologically and, while most of them failed to yield an organism, an aerobic, spore-forming type was secured from three of the cans. This organism was apparently not the primary cause of coagulation in the outbreak, but its general action on evaporated milk was of interest from the standpoint of showing the changes that microorganisms may bring about in the product.

METHODS

Opening cans. The cans, either for culturing or inoculation, were opened as follows: A small area on the top of the can was covered with concentrated HCl. After several minutes this was driven off, usually with a hot soldering iron, which carried a small amount of solder that was deposited on the tin and provided an excellent surface for resealing. A nail that had been thoroughly heated in a flame and cooled was then driven through the tin in the prepared area.

Culturing cans. A can was commonly cultured by means of a sterile capillary pipette.

Inoculation of cans. A can was inoculated with a capillary pipette when the inoculating material was liquid, otherwise with a needle.

EXPERIMENTAL

Preliminary cultural tests

In order to determine whether or not an organism could readily be secured from the defective milk, several cans were cultured, using (a) beef infusion agar slopes, (b) litmus milk, (c) litmus milk which was heated and cooled just before inoculation and sealed with a mixture of vaseline and paraffin immediately after, and (d) brain medium which also was

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heated and cooled just before inoculation. The amount of inoculating material used for a culture varied widely, smaller quantities being regularly employed with the agar than with the other media; in some instances as much as one cc. of the material was used in a tube of litmus milk. The cultures made from a can of milk were commonly divided into three groups and held at 21° C., at 37° C., and at 50° C.; as a rule comparatively few cultures were held at 21° C. because the history of the defect indicated it had developed at comparatively high temperatures. The preliminary cultural tests yielded only negative results.

The condition of the milk cultured varied a great deal. Some of the cans contained curd in such small amounts it could be detected only by straining the milk through gauze, in others there was a solid curd that had been more or less broken up, presumably during transportation, while in still others chunks of soft curd were floating in liquid milk. The milk from all of the cans had a normal odor, flavor, and acidity and was objectionable only because of the change in consistency.

At the time of culturing, curd from each can was spread on a slide, stained and examined under the microscope. Extended search failed to show the presence of organisms in any of this material.

Although the results obtained in the early cultural and microscopic examinations did not indicate the presence of organisms, bacteriological studies were continued because the defect had certain characters suggesting bacterial action, especially the appearance only after a holding period. Additional cans were cultured, using the general methods already given. Since the type of change suggested aerobic rather than anaerobic organisms, some of the beef infusion agar slope cultures were made in large test tubes (about 35 mm. in diameter) in order to make possible the use of large quantities of inoculating material. Occasionally, when only small amounts of curd were present, the milk was filtered through sterile gauze and the curd used for inoculation. Only negative results were secured until the examination of two cans which were sent from the condensery as the result of a request for cans from the last batch of milk showing spoilage. Each of these yielded an organism, apparently in pure culture, and from the microscopic and cultural studies the organisms from the two cans appeared to be the same. The organisms were secured on agar slopes and also in litmus milk cultures; it was evident, however, that they were present in the evaporated milk in very small numbers since only a portion of the cultures showed growth. With one of the cans growth was secured at both 37° C. and 50° C., while with the other growth was secured at only 37° C. Curd from the two cans yielding organisms on culture failed to show organisms microscopically in the examinations carried out at the time of culturing.

Preliminary inoculation experiments

Preliminary inoculation experiments were carried out with the strains isolated, using cans of evaporated milk held at 37° C. Each of the strains produced coagulation in the milk. With comparatively short incubation periods—from five to fifteen days—this was evident in cans which were disturbed very little after inoculation (a) as a distinct ring of curd along the wall of the can at the surface of the milk, (b) as small masses of curd adhering to the top of the can, and (c), in some instances, as masses of floating curd or as a distinct layer of curd over the surface of the milk. The odor, flavor, and acidity of the milk were normal. There were conspicuous differences in the time required for coagulation in the different trials. With incubation periods of several weeks or months the milk was usually completely curdled; the shortest incubation period noted as giving complete curdling was thirty-five days. Commonly there was a solid mass of curd with no whey but when the cans had been shaken occasionally during the incubation period the curd was more or less broken up and whey liberated. The odor and flavor of the completely curdled milk were commonly normal although when the incubation was continued for several months the flavor sometimes suggested bitterness. The striking point shown by the inoculation experiments was that the strains isolated could bring about complete coagulation of evaporated milk in cans without changing the odor or flavor. With long incubation periods at 37° C. uninoculated control cans of milk commonly showed considerable coagulation, which in the case of undisturbed cans was present at the surface of the milk and was most pronounced next to the tin; often there was also a change in the flavor of the milk.

When the inoculated cans were opened, microscopic examinations and culutural tests were commonly made. For a short time after inoculation organisms were found microscopically but always in small numbers. As the incubation period increased organisms could not be found microscopically; in a number of instances spore stains were made on the milk but these also failed to show the organisms. Cultures were positive except in a very few instances.

Coagulation also occurred in inoculated cans of milk held at temperatures below 37° C. but was conspicuously slower than that at 37° C.

Additional cultural tests

Several additional cans of evaporated milk from the batch yielding the organism were examined microscopically and culturally. All were negative microscopically and all but one was negative culturally. This yielded an organism which showed the same general characters as the cultures previously secured and in cans of evaporated milk it produced the same general change; in both unconcentrated and evaporated milk, however, it brought about a change somewhat more slowly than the other cultures.

The isolation of an organism capable of producing coagulation in evaporated milk from three cans of the defective product led to the culturing of many additional cans from various batches. Since the cultures made with small amounts of inoculating material had been so largely negative, a different procedure was used. A can of milk to be studied was opened, a rather large quantity of the milk transferred to one or more large test-tubes (usually 75 cc. of the milk to each of two test tubes) by means of a pipette to which a fine tube was attached with rubber tubing and the can then resealed with solder; incubation was at 37° C. Air was thus supplied to all of the milk originally in the can so that if a lack of oxygen had stopped the growth of an organism it should again find conditions favorable for development.

None of the attempts yielded an organism and the evidence, accordingly, indicated that the type isolated was present in only certain of the cans from one batch of evaporated milk. In a number of instances the milk transferred to the tubes and that remaining in the can firmed up when held at 37° C.; however, microscopic and cultural examinations failed to show the presence of an organism. This change occurred primarily with milk from cans showing considerable soft curd when first opened.

Detailed inoculation experiments with evaporated milk

The preliminary inoculation experiments showed the general type of change produced in cans of evaporated milk by the three strains isolated. When evaporated milk was transferred to sterile test tubes under aseptic conditions and inoculated with one of the strains the milk curdled rapidly; both microscopic examinations and cultures showed large numbers of organisms and the odor and flavor of the milk were distinctly objectionable. These results suggested that if the air supply were increased the changes produced in inoculated cans would be more rapid and extended.

Trials were carried out in which two cans of evaporated milk were inoculated with equal amounts of a milk suspension of one of the strains and the cans then sealed and thoroughly shaken to distribute the organisms added. One of the cans was reopened, a portion (usually 25 or 50 cc.) of the milk withdrawn and the can resealed. The two cans were incubated at 37° C. and occasionally shaken slightly in order to determine whether or not coagulation had occurred. The partly emptied can regularly firmed up very rapidly while the full can did not. Later the cans were cultured on beef infusion agar slopes and the tops removed to observe the extent of the coagulation. The cultures from both cans gave abundant growth. With a comparatively short incubation period the full can showed a limited amount of coagulation while the partly emptied can was firmly curdled, much firmer than many of the cans from the outbreak. The milk from the full can showed a small number of organisms microscopically soon after inoculation while with long incubation periods none could be found; the odor and flavor were normal unless the incubation period was very long. The milk from the partly emptied can showed many organisms and the odor and flavor were distinctly objectionable. Essentially the same results were secured when the two inoculated cans were allowed to stand several days at 37° C. before milk was removed from one of them. The trials show that with a greater air supply the changes produced in cans of evaporated milk by the strains isolated are the same as those produced in evaporated milk in test tubes.

The relationship of the air space in a can of evaporated milk to the extent of the change produced by the strains isolated was further studied by using several cans in each series and removing various, but comparatively small amounts of milk, from a number of them. The partly emptied cans regularly showed more of a change than the full cans and the more milk removed the more extended was the change. This relationship is shown in Fig. 1.

The influence of air on the change produced in evaporated milk inoculated with the organism was also evident in comparisons of sealed and unsealed test tube cultures. The test tubes were sealed by cutting them off about one inch above the surface of the inoculated milk, forcing a sterile cork almost to the surface of the milk and then pouring on sealing wax. In some of the trials uniform inoculation of the milk in the sealed and unsealed tubes was secured by inoculating a can of milk, shaking thoroughly, and then pipetting milk to the test tubes while in other trials the tubes of evaporated milk were inoculated directly from an agar slope culture. The unsealed tubes regularly coagulated before the corresponding sealed tubes; as a rule the sealed tubes required at least several days and commonly the coagulation definitely began at the surface.

Another method of showing the relationship of air supply to the change produced in evaporated milk consisted of inoculating two cans, resealing them with solder and then punching a hole in one of the cans and soldering over this opening a sterilized brass tube, about two inches long, that had a cotton stopper in the upper end. The changes occurring in the two cans were very different. While the milk in the sealed can showed the usual rather slow coagulation, no objectionable odor and flavor and very few organisms microscopically, the milk having the air supply coagulated very rapidly, developed an objectionable odor and flavor and showed enormous numbers of bacteria microscopically. In certain instances there was a membrane made up of organisms over the milk in the can with the air supply.

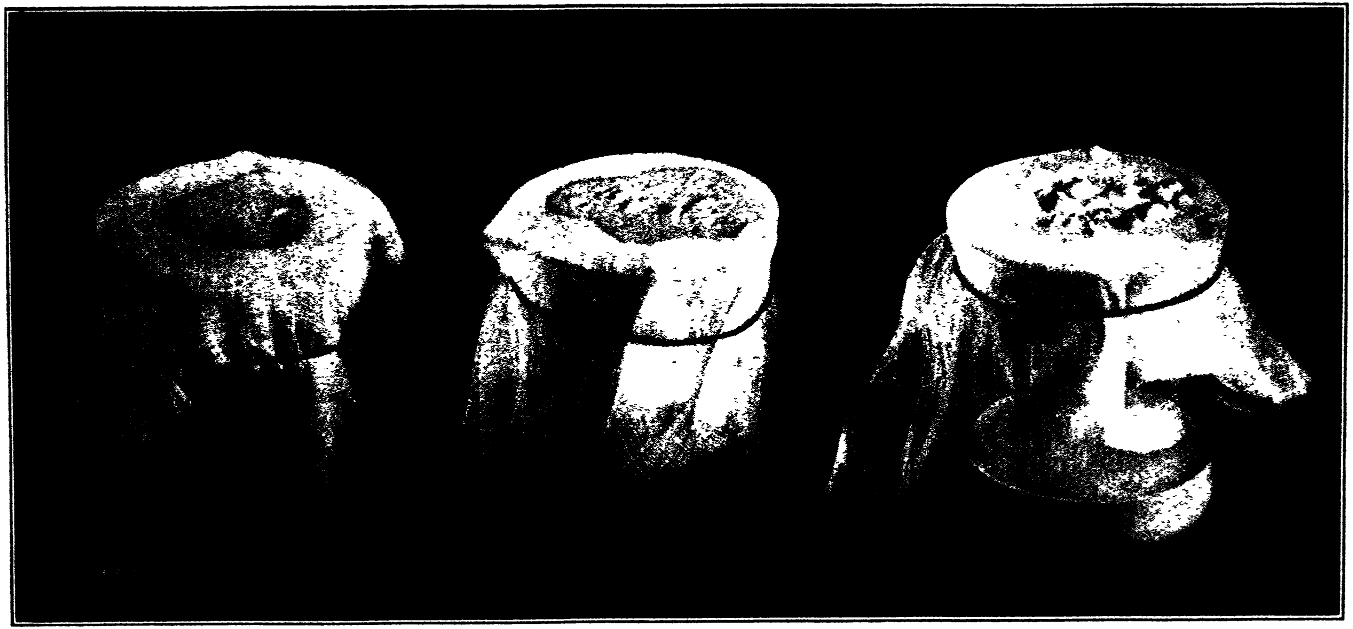

FIG. 1. Effect of air supply on coagulation in cans of evaporated milk inoculated with the organism isolated. Incubation six days at 37° C.

Left = full can.

Middle = five cc. of milk removed at time of inoculation.

Right = twenty cc. of milk removed at time of inoculation

Odor and flavor of all milk normal.

Additional evidence suggesting that the organism isolated was not the primary cause of the coagulation

While the cultural tests indicated that the organism isolated was present in only certain cans of the one batch of evaporated milk, and apparently, was not the primary cause of the coagulation, the development of a condition essentially like that noted in the outbreak—that is, coagulation with no abnormal odor or flavor—when the organism was inoculated into normal cans of milk suggested that it may have died in the milk that failed to yield it. Accordingly, attempts were made to secure additional evidence as to whether or not the organism had been active in the milk.

Survival of the organism in inoculated evaporated milk. The survival of the organism in cans of inoculated evaporated milk was studied by inoculating series of cans, incubating them at 37° C. and then culturing a can from each series on beef infusion agar slopes after various holding periods. The results showed that the organism remained alive for extended periods in evaporated milk into which it had been inoculated and could be recovered for at least eight or ten months; occasionally a can would give negative results when cultured but a later trial on a can from the same series would give positive results. Since many of the cans of milk from the outbreak were examined within three or four months of the date of packing it does not seem probable that the organism had been present in them and died.

Soluble nitrogen in inoculated evaporated milk. The soluble and amino nitrogen in cans of inoculated evaporated milk were studied and comparisons made with the soluble and amino nitrogen in cans of milk from the outbreak. The general procedure was to transfer the contents of a sixteen oz. can to a flask, add two cc. of glacial acetic acid, heat in a bath of boiling water with the container stoppered to prevent evaporation, cool and filter; the total nitrogen and amino nitrogen in the whey were then determined. Milk which coagulated in cans following inoculation with the strains isolated regularly showed higher soluble and amino nitrogen values than that in uninoculated cans. The increases over the controls were largest in the milk that had been inoculated for considerable periods but were very definite in milk inoculated for such short periods that only a small portion was curdled. The soluble and amino nitrogen values on cans of milk from the outbreak were essentially the same as on normal evaporated milk. Milk from the batch which yielded the organism was not available for nitrogen determinations. The results showed that the organism isolated very quickly caused a definite increase in the soluble and amino nitrogen in evaporated milk. Since no such an increase had occurred in milk from the outbreak, it appears that the organism was not responsible for the spoilage.

Composition of gas in cans of coagulated milk. The composition of the gas in cans of milk that coagulated following inoculation with the strains isolated and also in cans from the outbreak was determined by collecting it over water and subjecting it to the usual absorption materials. Oxygen and carbon dioxide were the constituents of primary interest. It was found that oxygen was generally absent in inoculated cans, in cans from the outbreak and also in normal cans of milk. The inoculated cans developed considerable quantities of carbon dioxide which were greatly increased by removing some of the milk so that the air spaces in the cans were larger. Rather large amounts of carbon dioxide were also found in cans from the outbreak but the same thing was true of uninoculated cans held for considerable periods at 37° C.

Effect of extended holding at 37° C. on normal evaporated milk

As already pointed out when uninoculated cans of normal evaporated milk were held at 37° C. for considerable periods, changes regularly occurred. These included (a) partial curdling of the milk which began at the surface next to the tin, (b) an increase in the color and (c) a change in the odor and flavor. The extent of the changes in a series of cans were closely related to the lengths of the holding periods and with a holding period of several months, the changes were pronounced; the removal of some of the milk from a can so that the air space was increased tended to accelerate the changes. Various lots of evaporated milk behaved differently and pronounced differences between brands were encountered. Many of the uninoculated cans held at 37° C. were cultured on beef infusion agar slopes and examined microscopically but organisms were never found. The results suggest that there are variations in the condition of the milk from the standpoint of its resistance to changes by heat. Such variations are known to exist when the milk is exposed to sterilizing temperatures and, accordingly, would be expected at lower temperatures also. If there are variations in the rate of coagulation of milk by heat, there may be comparable variations in the rate of coagulation by an enzyme; this would explain the variations in the time required for coagulation when the organism isolated was inoculated into different lots of evaporated milk.

The outbreak of coagulation supplying the milk studied occurred at a time when the atmospheric temperatures were very high so that a tendency to an instability to heat would have been much more likely to result in coagulation than at other seasons.

Identity of the organism isolated

The three strains of the organism isolated were studied morphologically, culturally and biochemically and identified as *Bacillus cereus*. Although *B. cereus* is described (1 and 4) as peptonizing milk without coagulation and the strains isolated definitely coagulated evaporated milk, organisms may bring about changes in evaporated milk that are somewhat different than those brought about in unconcentrated milk. Skimmilk cultures of *Bacillus ichthyosmius* (2) have a digested appearance while evaporated milk cultures do not; presumably the high percentage of solids in the evaporated milk prevents the digestion that occurs from changing the general appearance of the milk. The organism isolated brought about rapid peptonization of skimmilk with very little suggestion of coagulation.

The isolation of an atypical *B. cereus* from evaporated milk has been reported by Kelly (3); the evaporated milk showed a custard-like clot on top but no other change. The organism was considered atypical because it produced a weak neutral clot in milk and gave a viscous growth on agar that was shiny rather than mealy. Kelly also isolated an atypical *Bacillus simplex* and an atypical *Bacillus megatherium* from curdled evaporated milk.

The Vermont Agricultural Experiment Station (6) reported that *Bacillus subtilis* was the organism figuring most actively in an outbreak of coagulation in evaporated milk in which there was a non-acid curdling, the curd later separating from the whey as a slimy mass. Other organisms were found in the spoiled milk but were not considered important. Morrison and Rettger (5) secured a relatively stable variant of *Bacillus vulgatus* in pure culture from spoiled evaporated milk from two widely separated sections.

Action of the enzyme produced by the organism isolated

Crude enzyme preparations were secured from the organism isolated by adding chloroform to milk cultures, shaking frequently during a period of several days and then filtering through a bacteria-proof filter. After culturing on beef infusion agar slopes to be certain the filtrates were free from organisms, the coagulating power of the preparations was tested.

The enzyme preparations curdled litmus milk in a few days at 37° C.; with continued incubation the curd became firm and eventually a liquid separated from it. Evaporated milk, either in cans or in test tubes, was also curdled rapidly at 37° C. by the enzyme preparations and with continued incubation the curd became very firm. In the control tubes of evaporated milk—that is, the tubes to which no enzyme preparation had been added—a settling out of the casein eventually occurred but the condition was very different from that in the tubes to which enzyme preparation had been added.

The effect of heat on the enzyme was shown in a number of trials by adding equal quantities of enzyme preparation to two tubes or cans of evaporated milk and heating one tube or can in boiling water for some time before incubating at 37° C. The milk in the unheated tube or can coagulated rapidly while that in the heated tube or can did not.

In some instances soluble nitrogen determinations were made on evaporated milk that had been coagulated by an enzyme preparation; these showed that the soluble nitrogen had been markedly increased.

SUMMARY

In a study of evaporated milk from an outbreak of coagulation, an organism was secured from three cans, all of which came from one batch of milk. This organism curdled cans of evaporated milk slowly at 37° C. with no change in the odor and flavor, unless the incubation period was extended, and could be found microscopically for only a short time after inoculation and then only in small numbers. If inoculated evaporated milk was provided with a satisfactory air supply by transferring it to test tubes, removing a part of the milk from a can or in some other way, the organism curdled the milk rapidly, produced an objectionable odor and flavor and could be found in large numbers microscopically.

Since the organism was found in only three of the many cans of milk examined it did not appear to be the primary cause of the outbreak. Additional evidence along this line is provided (a) by the survival of the organism in inoculated milk at 37° C. for extended periods so that it is improbable the organism had died in the milk failing to yield it and (b) by an increase in the soluble and amino nitrogen in evaporated milk that coagulated following inoculation with the organism while the soluble and amino nitrogen in milk from the outbreak were the same as in normal evaporated milk.

Normal cans of evaporated milk showed changes on extended incubation at 37° C.; these included partial curdling, an increase in color and a change in the odor and flavor.

The organism isolated was identified as *Bacillus cereus*. The striking point in connection with its action in cans of evaporated milk was the coagulation with no change in the odor and flavor, unless the incubation period was extended.

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THE DETECTION OF INEFFICIENTLY PASTEURIZED MILK BASED ON A MODIFICATION OF THE NEW ROTHENFUSSER TEST

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INTRODUCTION

There is a recognized need for some test which can determine, with a fair degree of accuracy, whether a milk has been properly pasteurized. Besides being able to determine whether a milk has been pasteurized the test should also detect whether the milk has been heated below the pasteurization temperature. In the past there has been a great deal of inefficient pasteurization, due either to the selection of unsatisfactory time periods or to a failure to secure the exposures desired. Very often such pasteurization gives milk of fairly satisfactory keeping quality so that the deficiency is not readily noted. Pasteurization that is inadequate is thoroughly misleading and dangerous, since protection is not provided where it is expected and the insurance which the label affords is without any basis.

Neither 142° F. nor 145° F. as indicated by the recording thermometers for the main body of milk will offset a temperature drop frequently as high as 8° or 9° F. and occasionally as high as 30° to 40° F. in the milk in cold pockets which are beyond the influence of the heating and agitating devices.

It is, of course, obvious that the mixture of foam and milk which leaves the vat at the end of the pasteurization process is not safely pasteurized due to the fact that the temperature of the air above the milk is frequently far below the temperature of pasteurization as was shown by Archibald, Whittaker and Leete (1). These workers found that the temperature of the foam was always lower than that of the corresponding milk. They found that the difference in temperature varied from 5° F. to 18.1° F.

In view of these facts, public health officials have come to a realization that commercial pasteurization, which has been generally accepted as a perfect barrier against milk-borne disease, has, in reality, numerous defects. From a survey of present practises in dairy laboratories, no test has been accepted and universally used for the detection of inefficient pasteurization. Bacteriological analyses have been the only means used for this purpose in many laboratories giving uncertain and practically worthless results.

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* The latter part of this work, that dealing with tests under actual commercial conditions, was done in cooperation with the Dairy Division of the Boston Health Department through the courtesy of Mr. F. E. Mott, milk inspector and chemist for the City of Boston.

S. Rothenfusser (2) recommends and describes a method for the detection of heated milk, that is milk heated to 70° C. or above, and a new method for the detection of pasteurization. These tests are based on the action of heat on certain milk enzymes, peroxidase in the first case and diastase in the latter.

REVIEW OF FORMER METHODS

In the past various tests have been proposed and some have been used for the detection of heated milks. Very few tests have been proposed for the detection of pasteurized milk. The Schardinger test (3) depends upon the decolorizing action of a reductase in milk. The original test was made with methylene blue, but later it was found that a small amount of formalin added made the test more sensitive. Raw milk will decolorize the reagent in less than 20 minutes, heated milk will take longer than 20 minutes. With milk heated below 65° C. the reaction is doubtful.

The old Rothenfusser test (4) depends upon the oxygen liberating power of peroxidase in the presence of a peroxide and the absorption of the oxygen by an oxygen acceptor which can give indication of the change by a color reaction as in the case of guaiacol. This test is essentially similar to the new method proposed by Rothenfusser for the detection of heated milk. This test produces a blue violet coloration with raw milk and no color if the milk has been heated to 80° C. or over.

Since benzidine in the presence of hydrogen peroxide is changed by peroxidase action into an azo-compound and a blue color is produced, the Benzidine test (5) has been used as a means of detecting heated milk. This test is of no value in testing for pasteurization.

Nurenberg and Lythgoe (6), carrying out tests to distinguish between heated and raw milks by the above reactions and basing their conclusions on the evidence obtained from the analyses of 2500 samples, are of the opinion that the Benzidine and Rothenfusser tests are of no value in the detection of heating below 75° C. and 80° C. respectively, and therefore fail in their practical value. The Schardinger test was able to detect milk heated at 63° C. for 35 minutes to milk heated at 75° C. These workers found that none of the above methods would detect commercially pasteurized milk three days after pasteurization and only the Schardinger test will detect it up to the second day and then only very feebly.

The catalase test (7) is based on the effect of heat on the catalase in milk. In the presence of perhydrol the catalase liberates free oxygen. If the milk is heated to 62° C. for a short time a small amount of gas will be liberated, but if the milk is heated to 62° C. for one hour or 66° C. for 30 minutes, or 68° C. for one minute no gas is liberated. The test is of no value if the milk is inoculated with large numbers of bacteria. The test is negative in the presence of very small amounts of raw milk.

The only test, as indicated by the literature, which has been proposed for the detection of inefficiently pasteurized milk is the microscopic test of Frost (8). This test involves the staining of the cells present in milk. The procedure consists of mixing the milk with an equal quantity of special methylene blue stain, allowing the mixture to stand and then centrifuging; the sediment is spread on a slide, air dried and examined under oil immersion. Raw milks show a dark background with unstained leucocytes as clear areas. In heated milks the leucocytes are well stained while the background is light.

Frost and Moore (9) applied the cellular test to 260 of the routine milk samples analyzed by the Chicago Health Department Laboratories during July, 1918. All of 47 samples of raw milk were correctly placed although 11 showed indication of being partly heated. Of 213 samples of pasteurized milk only 4% were incorrectly placed, they report. Dr. K. Bailey and F. E. Mott, of the Boston Health Department Laboratories, have informed me, on the other hand, that this test as well as others which have been applied in their laboratories to show the extent of heating of milk at or about the pasteurization temperature have been entirely unsatisfactory.

NEWLY PROPOSED METHODS

New Rothenfusser Method (for Heated Milk) (2) :—

The lead serum of the milk is prepared by the addition to 100 c.c. of the sample the minimum excess of basic lead acetate solution (5 to 6 c.c.) required to produce complete precipitation. The mixture is shaken well, centrifuged and the clear serum should then contain no fat, casein, phosphates or citrates, but only chlorides, sulphates and acetates of sodium, potassium, calcium, magnesium and a trace of lead. The pH value is 6.6–6.7, and the acidity is equivalent to about 1 c.c. of 0.25 NaOH solution, and in serum from fresh milk both peroxidase and diastase should be present.

For milk heated at a relatively high temperature p-tetrol sulphite (stable in the dark) is used as a reagent.

Preparation:—Add 1.45 c.c. of a fresh 20% solution of sodium bisulphite to 100 c.c. of a mixture containing 1 gram of p-phenylenediamine hydrochloride in 12 c.c. of water, and 4 grams of crystalline guaiacol in 100 c.c. of 96% alcohol, diluted to 150 c.c. with 96% alcohol.

Test:—To 10 c.c. of the serum or milk are added 4 drops of 3% hydrogen peroxide and 10 drops of the reagent. A blue color shows the absence of heating above 80° C. The production of no color indicates heating above 80° C.

Rothenfusser Pasteurization Test (2) :—

A stable standard starch solution is prepared by grinding 10 grams of soluble starch (Merck & Co., according to Lintner) with 10 c.c. of water, and adding 500 c.c. of boiling water. The mixture is boiled gently for 10 minutes, (using an asbestos protector), 150 c.c. of pure glycerin (sp. gr. 1.23) is added and a clear solution should result. Boiling is continued for 10 minutes, then 6 grams of sodium chloride in 50 c.c. of water is stirred in and 5 c.c. of 0.25 N NaOH is added. The hot mixture is filtered, 250 c.c. of 95% alcohol is added in 50 c.c. portions and the mixture diluted to 1000 c.c. with boiled water, cooled and allowed to stand for a day or two. The supernatant liquid is then decanted from any sediment and placed in bottles which are then heated in a water bath at about 65° C. (not over) for 30 minutes. The starch is then ready for use in the test.

Test:—30 c.c. of the milk are shaken with 1.6 c.c. of lead reagent in a centrifuge tube, 2 c.c. of hydrochloric acid-free chloroform are added, and the mixture again shaken and centrifuged. The clear serum (10 c.c.) is then mixed with 1 c.c. of the starch and after 15 hours incubation at room temperature or 3½ hours at 40° C. 1.5 c.c. of the mixture is poured into a small tube and 1.5 c.c. of a 0.002 N iodine solution is added. A yellow color indicates heating insufficient to destroy the diastase. Red indicates the partial destruction of the diastase and blue the complete absence of active diastase.

EXPERIMENTAL DATA

In order to determine the influence of the temperature of pasteurization on these tests a quantity of raw milk of known purity was obtained. Numerous one pint portions were heated gradually in a water bath to the various temperatures listed in table 1 and held at that temperature for a period of 30 minutes. The milks were subjected to both the heated milk and pasteurization tests.

TABLE 1

Effect of Temperature on the Tests

TEMP. OF PAST.	HEATED MILK TEST	PASTEURIZATION TEST
20°C.	Blue	Yellow
40°	Blue	Yellow
50°	Blue	Yellow
55°	Blue	Yellow-gray
56°	Blue	Red-violet
58°	Blue	Violet
60°	Blue	Violet
63°	Blue	Violet
65°	Blue	Blue
70°	Blue	Blue
75°	Faintly Blue	Blue
80°	No Color	Blue

From the foregoing table it can easily be seen that the heating of milk cannot be detected by the peroxidase test unless the heating exceeds 80° C.

The diastase test, however, could detect the pasteurization of milk very readily as was shown by the marked difference in the color after the milk had been pasteurized. It was, however, impossible to detect hardly any difference in color between milk heated at 58° C. for 30 minutes and that heated at 60° C. for the same period, under ordinary conditions with the naked eye. However, with the aid of a tintometer, which is a simple device consisting of an elongated box with an eyepiece at one end and two rectangular openings at the other, one for the solution to be examined and one for the standard solution or the standard glass slides used for matching colors, light being reflected through the openings by a square piece of unglazed glass mounted on a standard, it was possible to show the preponderance of red color produced by the erythrodextrin upon the addition of the iodine when the milk had been heated to 58° C. over that when heated to 60° C.

The colors produced were not very clear and did not lend themselves to simple analysis with the tintometer. In order to produce a clearer solution a weaker solution of iodine was used in the test. An experiment was carried out using a 0.001 N iodine solution with the following results when the colored solutions were examined by means of the tintometer.

TABLE 2

Pasteurization Test using 1 c.c. Starch and 0.001 N Iodine

TEMP. OF PASTEURIZATION	TIME	BLUE	RED
56° C.	30 minutes	4.0 tint units	5.0 tint units
58°	30 "	4.5 "	4.5 "
60°	30 "	5.0 "	4.0 "
60°	20 "	4.3 "	4.7 "

From Table 2 it can be seen that the ratio of blue to red increases directly with the increase in temperature of pasteurization. Also the experiment shows that the ratio decreases with a decrease in the period of holding. From the results of this experiment it can be seen that an excess of starch solution is being used in the original test.

Therefore the following experiment was undertaken to determine whether the blue coloration produced by the excess of starch upon the addition of the iodine solution could be eliminated and therefore not obliterate the red color produced by the erythrodextrin upon the addition of iodine.

For these tests both 0.001 N and 0.002 N iodine solutions were used and to the 10 c.c. portions of the lead serum were added both 0.5 c.c. and 1 c.c. portions of the starch solution. The milks were heated to temperatures of 55° C., 56° C., 58° C., 59° C., 60° C., and 62.8° C. and held at that temperature for 30 minutes. One sample was heated at 60° C. for 20 minutes. Table 3 shows the results of this experiment.

TABLE 3

The Effect of Varying the Amount of Starch Solution and the Concentration of the Iodine Solution

TEMPERATURE OF PASTEURIZATION	0.5 C.C. STARCH		1 C.C. STARCH	
	.001 N I_2	.002 N I_2	.001 N I_2	.002 N I_2
55° C.	Orange	Brown	Red-violet	Brown
56°	Orange	Gray-brown	Red-violet	Violet
58°	Red-orange	Yellow-brown	Blue-violet	Blue-violet
59°	Red-orange	Yellow-brown	Blue-violet	Blue-violet
60°	Red-violet	Dirty Brown	Blue-violet	Blue-violet
60.8°	Blue-violet	Dirty Brown	Blue-violet	Blue-violet
60° C./20 min.	Red-orange	Gray-brown	Blue-violet	Blue-violet

From table 3 it can be seen that by the use of a 0.001 N solution of iodine and only 0.5 c.c. of the standard starch solution a distinct change in color occurs between 59° C. and 60° C. At 60° C. the test shows a blue coloration for the first time and it can be concluded that the production of any blue upon testing a milk by this modified test indicates adequate pasteurization. The appearance of a red or orange color indicates insufficient time or temperature exposure.

By means of a simple comparator block, having one cylindrical compartment for the small test tube containing the colored solution and a slot parallel to this for the standard glass slides, the colors being compared by looking through two holes which intersect the compartments and allow the light to pass through, it was possible to distinguish the smallest amount of blue.

In extensive trials carried out by a group of investigators (10) working with commercial equipment bovine tubercule bacilli were destroyed at 58.9° C. when held for 30 minutes. Milk heated at 60° C. for a period of 30 minutes has generally been accepted as safely pasteurized, therefore the following experiment was carried out to determine the exact color produced by a milk so treated when subjected to the modified test.

The milks were pasteurized in the laboratory and tested, using the 0.001 N iodine and 0.5 c.c. of the starch solution. The resulting colored solutions were examined by means of the Lovibond tintometer, previously described, and the color standardized. It was found that the color was similar to that produced by the combination of 1.15 standard red tint units and 1.00 standard blue tint units when observed by transmitted light.

DETECTION OF CONTAMINATION BY UNDERPASTEURIZED AND RAW MILK

Due to the fact that it is well known that small amounts of poorly pasteurized milk often, due to poor apparatus, cold pockets or an excess of foam, contaminate the entire body of milk the following experiment was performed to determine the practicability of the modified test as a means of detecting small amounts of underpasteurized milk in pasteurized milk. For this purpose milk heated at 51° C. and 56° C. for 30 minutes was added to commercially pasteurized milk in the amounts listed in table 4 and the modified test was applied.

TABLE 4
Addition of Underpasteurized Milk to Pasteurized Milk

TEMP. OF ADDED MILK	MILK ADDED			
	0%	1%	2%	3%
51° C.	Red-violet	Orange	Orange	Orange
56° C.	Red-violet	Red-violet	Red-orange	Orange

The tests carried out in this experiment show that the modified test is of sufficient delicacy to detect one per cent of milk heated at 51° C. for 30 minutes and two per cent if heated at 56° C. for the same period.

It is, of course, necessary that the test be able to detect the smallest amounts of raw milk since under certain conditions cases have occurred where the pasteurized product has been contaminated by the addition of raw milk. Therefore tests were carried out to determine the amount of raw milk that could be detected in pasteurized milk by the modified test.

TABLE 5
Addition of Raw Milk to Pasteurized Milk

RAW MILK ADDED		
0%	1%	2%
Red-violet	Orange-red	Yellow

Here it can be seen that so small an amount of raw milk as one per cent added to the pasteurized milk can easily be detected by the test and the presence of as much as two per cent gives a reaction similar to that for raw milk.

For the purpose of determining whether the reaction for raw milk returned after allowing pasteurized milk to stand for a few days, the following experiment was undertaken:—Samples of freshly pasteurized milk of good quality were obtained from the pasteurizer of a reliable milk concern. The milk had been pasteurized at a temperature of 145° F. for a period of 30 minutes. The samples were kept in the refrigerator and a new sample was examined daily. The modified test was applied and it was found that even after 5 days the heating could be detected. The test was not carried out after the fifth day.

In conformance with the suggestion of Rothenfusser the modified test was carried out using an incubation temperature of 37.5° C. rather than room temperature. It was found that the modified test gave results similar to those obtained in the previous experiments when the test solutions were incubated at 37.5° C. for a period of 4 hours.

THE MODIFIED TEST UNDER PRACTICAL CONDITIONS

The following experiment was carried out to determine the value of the modified test under actual commercial conditions. Complete data have been obtained on 36 samples of pasteurized milk taken under the supervision of the Boston Health Department and 18 pasteurized milks obtained by the author, as well as 36 samples of raw milk collected for this work. In all 90 samples were examined.

Modified Test; 30 c.c. of the milk were shaken thoroughly in a centrifuge tube with 1.8 c.c. of basic lead acetate, 2 c.c. of hydrochloric acid-free chloroform added, the mixture again shaken and centrifuged for about 15 or 20 minutes. Ten c.c. of the clear serum (unfiltered) were then mixed with 0.5 c.c. of the starch solution and incubated at 37.5° C. for a period of 4 hours. After incubation 1.5 c.c. of the mixture was poured into a small tube and 1.5 c.c. of a 0.001 N iodine (I–KI) solution added. The 0.001 (I–KI) solution was prepared by adding 1 c.c. of a solution containing 12.7 grams of resublimed iodine and 18 grams of pure potassium iodide (KI) in 1000 c.c. of water to 99 c.c. of water. The colored solution was immediately placed in the comparator block and compared with a standard slide of 1.15 red tint units and 1.00 blue tint units.

The appearance of blue in excess of the standard indicates heating above 60° C. (140° F.) for 30 minutes. A distinct red or orange indicates heating below the pasteurization temperature, insufficient holding or the presence of small amounts of raw or poorly pasteurized milk. A yellow color indicates raw milk or milk heated not above 50° C.

Of the 36 milks taken and turned in by the inspector as pasteurized all indicated that they were properly pasteurized by the modified test. By means of the comparator block those milks pasteurized at 145° F. could unhesitatingly be distinguished from those pasteurized at a lower temperature due to the marked increase in the blue when the milk is treated at the higher temperature. All of these milks were obtained from milk concerns producing a milk of good quality.

Of the 18 pasteurized milks obtained by the author, 17 could be immediately detected as adequately pasteurized milk while the other sample, which was a milk pasteurized in the bottle, indicated that it had been heated slightly below the pasteurization temperature. This was due, in the opinion of the author, to the process of pasteurization used. However a number of samples of milk pasteurized in the bottle were tested and indicated that they had been adequately pasteurized.

Of the 36 raw milks examined all gave the yellow color diagnostic of a raw milk. In every case the test was of such a nature as to be unhesitatingly recognized as raw milk.

From the limited number of tests carried out on actual commercial samples it can be seen that the test was able to detect the state of the milk in all of the cases. One milk reported as pasteurized but found to be poorly pasteurized was a milk pasteurized in the bottle and the chances are that the milk may have been contaminated by a small amount of underpasteurized milk from the center of the bottle. Therefore the modified test, as indicated by this experiment, proved to be absolutely accurate.

DISCUSSION

Since this test depends on the effect of heat on the diastase present in milk observations were made to see whether diastase was actually present in those milks examined. Using the 36 samples of raw milk of which 18 were certified and 18 were raw market milk all showed the presence of diastase. With all the samples examined in carrying out the tests under actual commercial conditions the test was positive. Basing conclusions on this work and that done by Rothenfusser and others it is safe to say that milk normally contains diastase.

All other tests based on the theory that enzymes, as a rule, in contradistinction to inorganic catalysts, are destroying at temperatures somewhat below 100° C., fail in the detection of inefficiently pasteurized milk due to the fact that the enzymes involved are not affected by the pasteurization temperature. On the other hand the hydrolysis of starch by diastase takes place in a number of steps the rate of reaction depending on the amount and activity of the diastase. In the vicinity of the pasteurization temperature the activity of the milk diastase is attenuated. Above the pasteurization temperature the diastase is destroyed. The soluble starch used in the test is converted to erythrodextrin, the erythrodextrin is further hydrolyzed to α and β achroödextrin, and these dextrins are finally completely hydrolyzed to maltose. These several products of hydrolysis of starch give different colors with iodine and explain the varying shades of blue, lavender and red observed when the hydrolysis of starch is followed by the addition of iodine. In the modified test the reagents have been so limited that the starch is entirely converted to the dextrins and maltose when acted upon by diastase heated just below 60° C. and gives a red or orange color upon the addition of iodine.

Due to the somewhat delicate nature of the modified test there are a few important and fairly simple precautions which ought to be taken in carrying out the test in order to obtain uniform results.

(1) Since the test depends upon the action of exceedingly small amounts of diastase in the milk the introduction of diastase from external sources will result in spurious conclusions. For this reason the author recommends,

although it is not entirely necessary if care is taken, the use of calibrated test tubes in order to avoid the use of pipettes, since the introduction of the distinctly hydrolytic enzyme, ptyalin, from the saliva will change the reaction. For measuring the starch glass stoppered burettes with fine bore could be used.

(2) All apparatus which comes in contact with the milk or the serum must be free from any acid. All the reagents employed must be acid-free. This precaution must be taken since small amounts of acid will cause the hydrolysis of the starch. Pure laboratory reagents may be used but simple tests should be carried out to detect the presence of any acid.

(3) In order to get uniformity of results very little time should be lost in examining the colored solution upon the addition of the iodine. It is necessary to observe the color immediately since the color tends to fade.

(4) Since the diastatic activity very slowly increases, it is advisable that the milks be examined not more than twenty-four hours after pasteurization.

Although these few precautions may appear to be very bothersome, in actual practice they can be observed by employing the customary amount of laboratory care. The author, in carrying out the experimental work of this paper, found it of practically no difficulty to observe these few simple precautions.

From the work done on the original Rothenfusser test for pasteurization in the first experiment it can be seen that this test for pasteurization does not serve satisfactorily as a means for detecting the degree of pasteurization. The second experiment using the weaker solution of iodine did, to a certain degree, indicate the degree of heating with the use of a tintometer. However, this modification of the test would not be satisfactory for practical use due to the fact that the cost of a tintometer and set of standard slides would be entirely prohibitive for the average dairy laboratory. Moreover, the time involved in the matching of the colors by this method is very long and this fact alone detracts from its value since little time should be lost in matching the color.

The modified test using the 0.001 N iodine solution and only 0.5 c.c. of the standard starch solution eliminates the use of this apparatus entirely and substitutes a very simple piece of apparatus which can be constructed very easily in the laboratory and with the aid of a standard slide equivalent to the combination of 1.15 red tint units and 1.00 blue tint units can detect whether milk has been heated adequately from a public health standpoint. If the test is to be carried out without regard for precision or on milks which have been heated above the minimum pasteurization temperature the use of the block and the standard glass slide is unnecessary and the production of any blue color in the test solution is a fairly accurate indication of adequate pasteurization.

In all of the tests carried out on actual commercial samples the use of the comparator block and the standard glass slide was unnecessary since almost all of these milks had been pasteurized at a temperature of about 144° F. as indicated by the recording thermometers. The positive test, as indicated by the production of blue, at this temperature is so characteristic that the test need not be carried further.

That the modified test has its advantages over those tests previously used is shown by the results obtained in the various experiments carried out in this work. In contradistinction to the microscopic test, milk heated very slightly below the pasteurization temperature could be easily detected and milk held at a satisfactory temperature but for an inadequate period of time could also be detected. Although the modified test could not detect such small amounts of raw milk in pasteurized milk as the catalase test is capable of (0.25 per cent), the modified test can detect as little as one per cent. Moreover, the modified test can detect small amounts of poorly pasteurized milk in the pasteurized products. As indicated by the literature none of the reported tests are sufficiently delicate for this purpose.

Unlike the Schardinger test and the catalase test the modified test is not markedly influenced by bacterial action. This was illustrated by the tests carried out on commercial samples. No precautions were taken to keep the milk clean, on the other hand, many of the tubes used for the collection of the samples were unusually dirty. The milk was kept in these tubes for a period of about 2½ hours before testing and from the results of the tests it can be seen that no action was produced by the bacteria. As indicated by the tests carried out to see how long after pasteurization the test was applicable it can be seen that the bacterial action is very slow.

CONCLUSIONS

(1) The Rothenfusser test for heated milk can detect milk heated at 80° C. or over but is unable to detect pasteurization.

(2) The new Rothenfusser test for pasteurization while being able to detect pasteurization is not sufficiently delicate to detect whether milk has been slightly underpasteurized or not.

(3) The test as modified, using 0.001 N iodine solution and only 0.5 c.c. of the standard stable starch solution, can detect milk heated below 60° C. for 30 minutes or milk heated at 60° C. for less than 30 minutes.

(4) The modified test can detect as little as one per cent of raw milk in the pasteurized product and can also detect small amounts of slightly underpasteurized milk in pasteurized milk.

(5) The modified test is not easily affected by bacterial action.

(6) The modified test produces, as was shown by actual commercial application, very satisfactory results in the detection of inefficiently pasteurized milk.

ACKNOWLEDGMENT

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FAT METABOLISM IN THE LACTATING GOAT

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In a recent study in this laboratory (1) it was shown that the removal of most of the fat from a ration by benzine extraction of the grain mixture and its replacement by an isodynamic amount of starch resulted in a lowered milk and fat yield, accompanied by a lowering of the fatty acids and cholesterol in the blood plasma.

In view of the results obtained it seemed desirable to study the effect of a more complete removal of the fat, involving the roughage as well as the grain. It also seemed desirable to get more specific proof that it was the fat itself of the benzine extract which was responsible for the changes noted. The question as to whether some specific fat was particularly concerned also arose in view of the work of Burr and Burr (2, 3) showing that certain specific fatty acids are essential for the growth of rats.

Since it seemed impracticable, because of the expense involved, to use cows in such studies which would involve the use of certain purified foods and vitamin concentrates, it was decided to test out the suitability of goats for the purpose. The present paper reports the results of our first series of studies with goats in which the experiments with the cows have been repeated at a lower fat level and in which a limited amount of data has been obtained with specific fats.

EXPERIMENTAL PROCEDURE

The plan of the experiment called for a ration consisting of a grain mixture from which the fat could be completely removed and replaced by either starch or an oil, and of a combination of ingredients of minimum fat content which could serve as the roughage portion. As a result of several preliminary experiments a combination consisting of 55 per cent of beet pulp, 25 per cent of corn cobs and 20 per cent of molasses was selected as this roughage portion. This combination was readily eaten by the goats in the amounts desired and contained only 0.63 per cent of ether extract of which presumably less than half was digestible fat.

The grain mixture was made up according to the following formula:

20 per cent wheat bran
12 per cent ground soy-beans
25 per cent linseed oil meal
28 per cent hominy feed
15 per cent cottonseed meal, 43 per cent protein

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An amount of the above sufficient to last through the whole experiment was mixed and one portion of it was set aside as the normal grain mixture. The other was extracted by the benzine process to provide the low-fat mixture to which could be added starch or oil in amounts isodynamically equivalent to the fat removed. The fat which was removed was replaced by an equicaloric amount of starch for the low-fat ration.

A linseed oil mixture was prepared by adding sufficient of this oil to the extracted feed so that the resulting mixture contained a percentage of fat equivalent to that of the normal grain mixture. A mixture containing cocoanut oil was similarly prepared. An opportunity was thus provided for studying whether the lower yields caused by the substitution of starch for the material extracted by the benzine was due to a lack of fat itself.

Linseed oil was used since it is a commonly occurring one in dairy feeds. Cocoanut oil was selected for comparative purposes to obtain preliminary information as to whether the nature of the oil, particularly its state of saturation, was in anyway significant. The desirability of obtaining data on this question was suggested by the recent work of Burr and Burr (2, 3) who have reported that a minimum amount of fat is necessary for the growth and health of rats and that only certain unsaturated fatty acids, notably linolic, are effective. Linseed has an Iodine Number of 175 to 202 and is rich in linolic acid. Cocoanut oil is much more saturated with an Iodine Number of 8 to 10 and with a minimum of linolic acid. The oils[1] used were refined products but not deodorized.

TABLE 1

Analyses of feeds

FEED	CRUDE PROTEIN PER CENT	CRUDE FIBER PER CENT	ETHER EXTRACT PER CENT
Beet pulp	8.50	20.00	1.00
Corn-cob meal	3.07	34.59	0.31
Molasses	4.00		....
Normal grain mixture	25.35	8.01	6.81
Low-fat grain mixture and starch	23.47	7.42	0.244
" " " " Linseed oil	25.35	8.01	6.81
" " " " Cocoanut oil	25.35	8.01	6.81

The analyses of the various feeds are shown in table 1. These various grain mixtures were fed in different periods to four goats as is shown in table 2 and the charts following.

The ration for each animal was calculated on the basis of the requirements for cows as set forth in the Morrison Standard. However, the amounts provided in each case at the start of the experiment were some 15 per cent in excess of those called for by the standard and these amounts were not changed throughout the experiment. In view of our lack of knowledge as to the requirements of goats and the possibility of a depressed digestibility with certain of the combinations, it seemed desirable to provide this presumably excessive allowance to make certain that the possibility of a deficiency of either digestible protein or total digestible nutrients could be dismissed in interpreting the results. The intakes of nutrients are shown in table 2.

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[1] These oils were supplied for this work through the courtesy of Spencer Kellogg & Sons, Inc., Buffalo, N. Y.

TABLE 2

Average daily intake of nutrients by periods

GOAT	PERIOD	DIGESTIBLE PROTEIN GMS.	TOTAL DIGESTIBLE NUTRIENTS GMS.	TOTAL ETHER EXTRACT GMS.
A	Normal	160	1020	49.8
	Low-fat	158	1048	6.9
	Normal	158	1013	49.7
B	Normal	160	1020	49.8
	Low-fat	124	850	6.2
	Linseed	143	904	43.4
C	Low-fat	153	1012	6.7
	Linseed	158	1013	48.4
	Low-fat	158	1048	6.9
	Cocoanut	158	1013	48.4
D	Low-fat	155	1018	6.7
	Linseed	158	1013	48.4

It is noted that the intakes of digestible protein and total digestible nutrients were substantially constant for a given animal in each period with the exception of goat B. In the first part of the low-fat period this animal refused a portion of its feed and thus the average figure for the period is low. During the subsequent linseed period it received the same amount it was eating at the close of the low-fat period. In both of these periods, however, the animal ate more than its requirements by the Morrison Standard.

It is noted in table 2 that during the low-fat periods the intake of ether extract was less than 7 grams per day of which presumably less than half was actually digestible fat, whereas the normal and oil rations supplied seven times as much ether extract which was principally fat and presumably highly digestible.

A typical day's ration for a goat receiving the normal or one of the oil rations was as follows: grain, 634 grams; corn-cob meal, 226 grams; beet pulp, 453 grams; molasses, 136 grams. On the low-fat ration the goat received the same amount of corn-cob meal, beet pulp and molasses with a sufficiently larger amount of grain to make up for the smaller amount of total digestible nutrients supplied due to the substitution of starch for fat. In addition to the ration as listed above, each animal received daily 25 grams of a mineral mixture consisting of equal parts of bone meal, limestone and salt, and 25 grams of extracted yeast. The latter was given to make certain of an ample amount of vitamin B for lactation.

In addition to the food intakes which have been referred to, the data obtained included the following: weights of animals, yield and fat content of milk, the total lipids, phospholipids and cholesterol of the blood plasma, and the saponification number and the iodine number of the milk fat. The phospholipids represent an additional determination not made in the work with cows by Maynard and McCay (1). It was included in view of the special importance attached to this group in milk secretion. It was determined as lipid phosphorus by the Denigès method (4) as modified by McCay (5). The phosphorus was calculated to phospholipid on the basis of the formula for lecithin, $C_{44}H_{90}NPO_{9+}4H_2O$. The other blood lipids were determined as in the earlier report (1). The iodine number was determined by the Hanus Method. The saponification number, also a determination not made in the previous study, was determined by official method of the Association of Official Agricultural Chemists.

RESULTS

The weights of the animals are shown in chart I. The curves are plotted on the basis of average values of two weights in each five-day period. It is noted that all of the animals gained in the course of the experiment. This is evidence that the rations fed were more than adequate for milk production in all cases. The heavy vertical lines on each weight curve indicates the point at which the goat ration was changed. The diet a goat received may be determined from charts II to V. It is observed that goat B

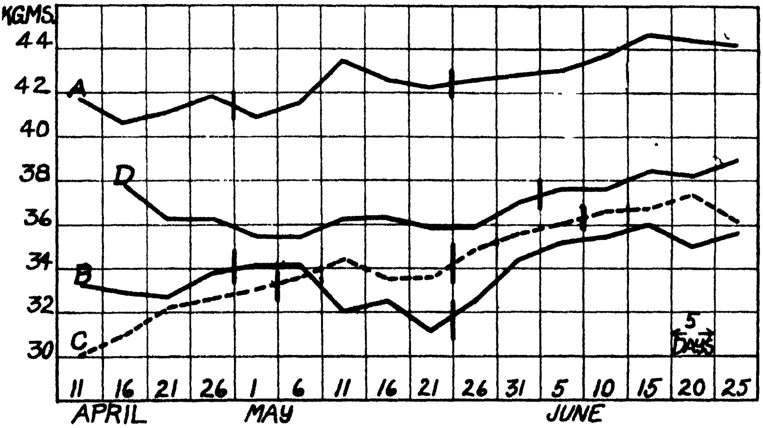

CHART I. BODY WEIGHT OF GOATS

dropped in weight after going off feed. Weight increased again when appetite picked up in the latter part of the low-fat and subsequent linseed period.

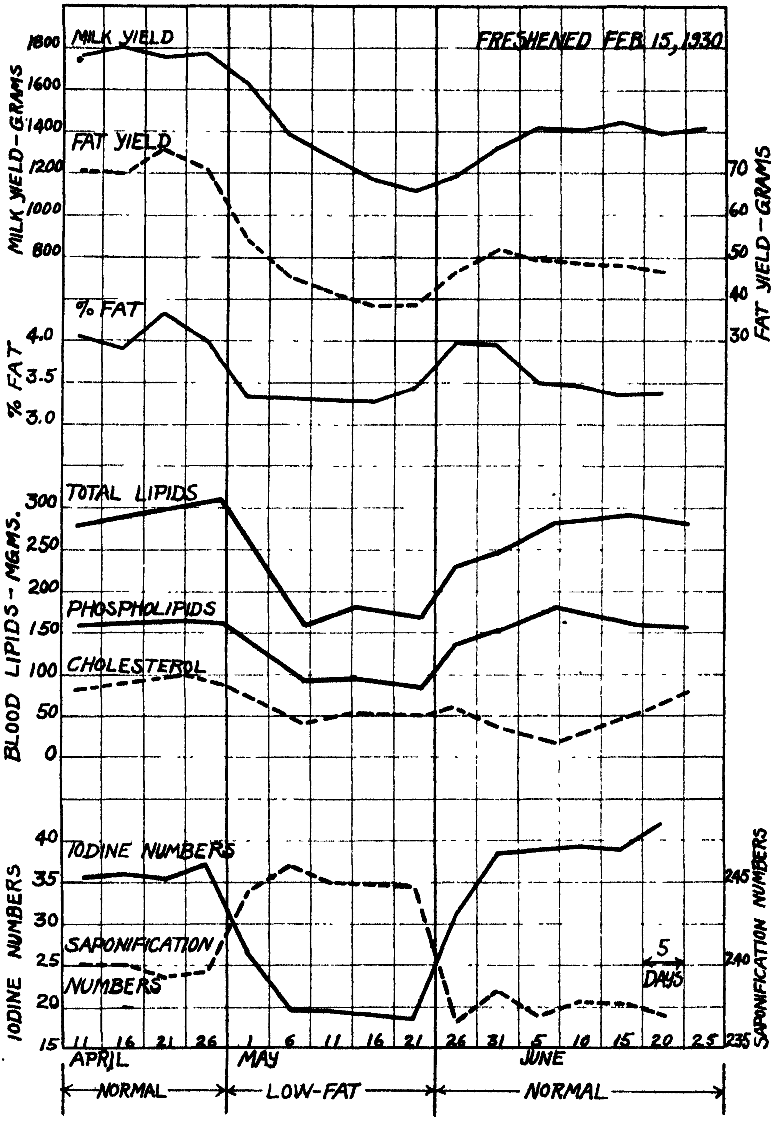

Chart II. Results Obtained with Goat A

The results of the milk and blood studies are presented in Charts II to V, one chart containing the data for each goat. In all the curves except those for blood lipids, each point is plotted in the middle of the 5-day period for which it represents an average value. The heavy vertical lines in the charts mark the times when a change of ration was made. These changes were made abruptly. The distinguishing feature of each ration is noted at the bottom of the charts.

The data for milk and fat yield will be discussed first. In considering these data it should be borne in mind that the normal trend of the curves would be downward with the possible exception of a short period at the start when the normal trend may have still been upward in view of the short time which had elapsed since freshening. It should also be noted that since the points of the curves are plotted in the middle of the five-day pe-

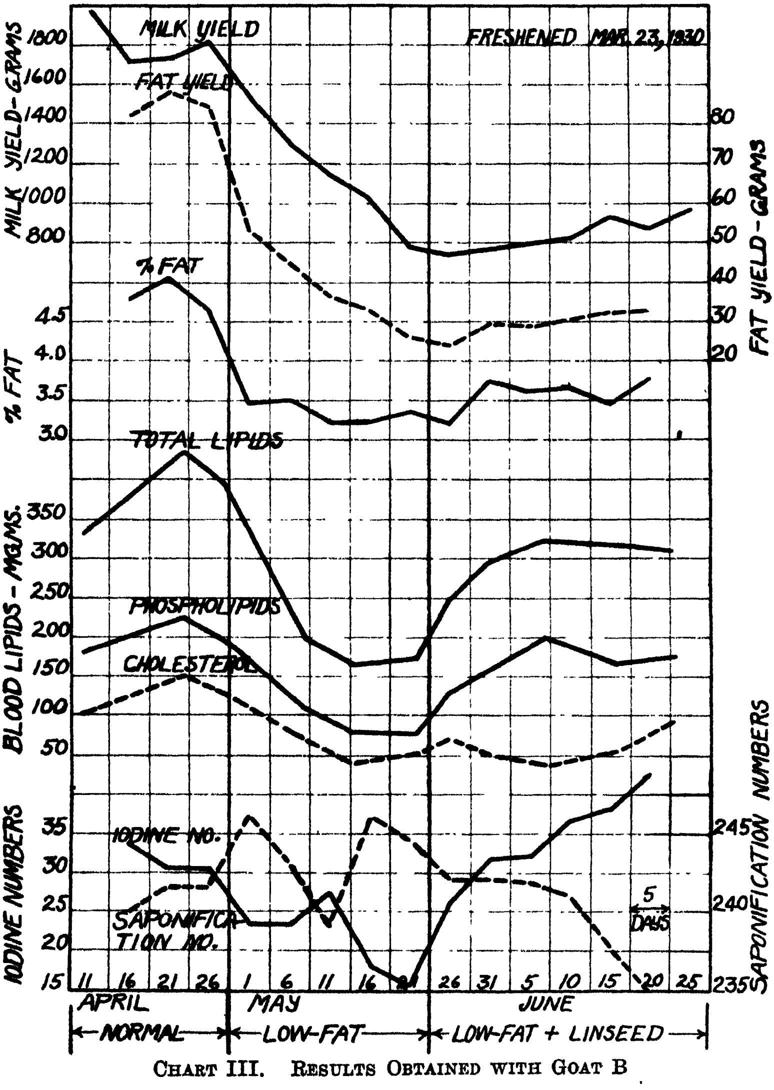

Chart III. Results Obtained with Goat B

riods any change in trend due to a change in ration should be looked for following the last point plotted for a given ration and not at the vertical line separating ration periods.

It is shown in chart II that Goat A was fed the normal grain mixture for 20 days, changed to the low-fat mixture for 25 days and then placed back upon the normal ration for 35 days. It is clear that the change to the low-fat mixture resulted in a lowered milk and fat yield which was followed by a rise when the normal mixture was restored. No definite trend is evident in the curve for fat percentage. This is the same picture that was found by Maynard and McCay (1).

In chart III a more marked drop is shown in yield of milk and fat during the low-fat period. The yields at the close of the period are less than half what they were during the period with the normal mixture. A sharp drop in fat percentage is to be noted during the first five days. Upon the substitution of linseed oil for the starch in the low-fat ration, the marked and continuous drop in yield of milk and fat is replaced by a small but continuous rise throughout the period. This is interpreted as a positive effect for the oil. The fat percentage tends to be higher during this last period but the data are too variable to be significant.

In chart IV there is a rise in milk and fat yield during the first part of the low-fat period. This is contrary to all our other data with both cows and goats. Perhaps this animal which had been fresh only 19 days when placed on experiment had not reached the peak of her lactation which normally occurs in the cow during the first 30 days after parturition. During this period it is recognized that underfeeding has much smaller effect than later. This is a possible explanation why the behavior here was different from what we have found in the other cases. It seems probable, particularly in view of the results for Goat D which are discussed later, that the drop would have occurred had the short period of observation been extended. These unexpected data in the low-fat period also make the results for milk yield during the linseed period difficult of interpretation. The fact that the milk yield remained constant could be interpreted as a favorable effect for the oil, provided the animal had passed through her peak period and that the normal trend would thus be downward. But if this was not the case, the oil may have had no effect. The results for per cent fat and fat yield are more definite since the trends are definitely upward during the period.

In the second low-fat period the usual marked fall in milk and fat yield is noted with a lesser drop in fat percentage. Upon the substitution of the cocoanut-oil ration the drop in milk yield is replaced by a rise. The drop in fat yield ceases but there is no rise because the trend of the fat percentage is downward. This one trial suggests a positive effect for the cocoanut oil on milk yield but the data are too limited to constitute proof.

Goat D (chart V) had been fresh only 10 days when placed on the experiment and, in view of the results obtained with Goat C, she was kept upon the low-fat ration for a longer time accordingly. For 20 days her milk and fat yield remained unchanged and then they dropped markedly throughout the remainder of the period. Upon the change to the linseed ration the downward trends in milk and fat yield cease and the percentage of fat rises. However the changes here are not marked.

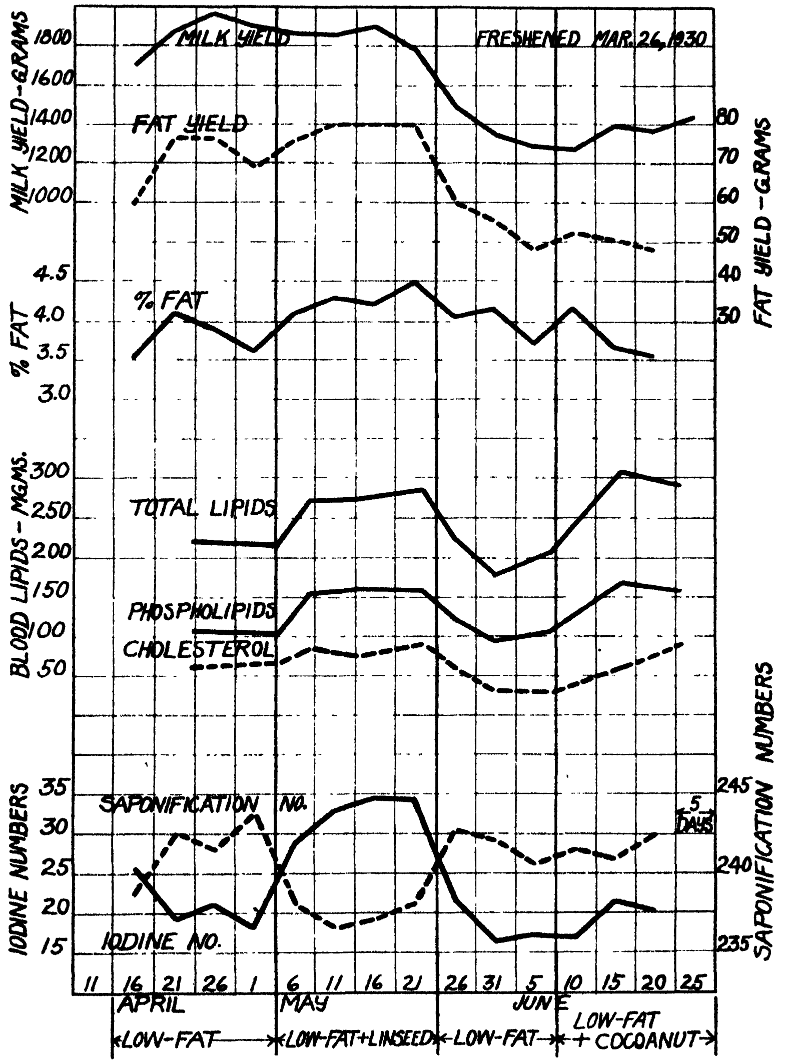

Chart IV. Results Obtained with Goat C

A striking feature of all the data for milk and fat yield is the very large and continuous decreases which occurred, with one exception, during the low-fat periods, despite the fact that each animal continued to receive a ration in excess of the requirements of her production throughout the period. These decreases range from 25 to 55 per cent for milk yield and 35 to 70 per cent in fat yield over periods from 15 to 40 days in length. They are much larger than have occurred in our cow experiments and presumably represent the effect of the more complete removal of the fat from the ration. The figures cited indicate that the fat yield decreased more than the milk which means that the fat percentage decreased also. This is a different finding from that obtained with the cow studies. In the latter a sharp drop in

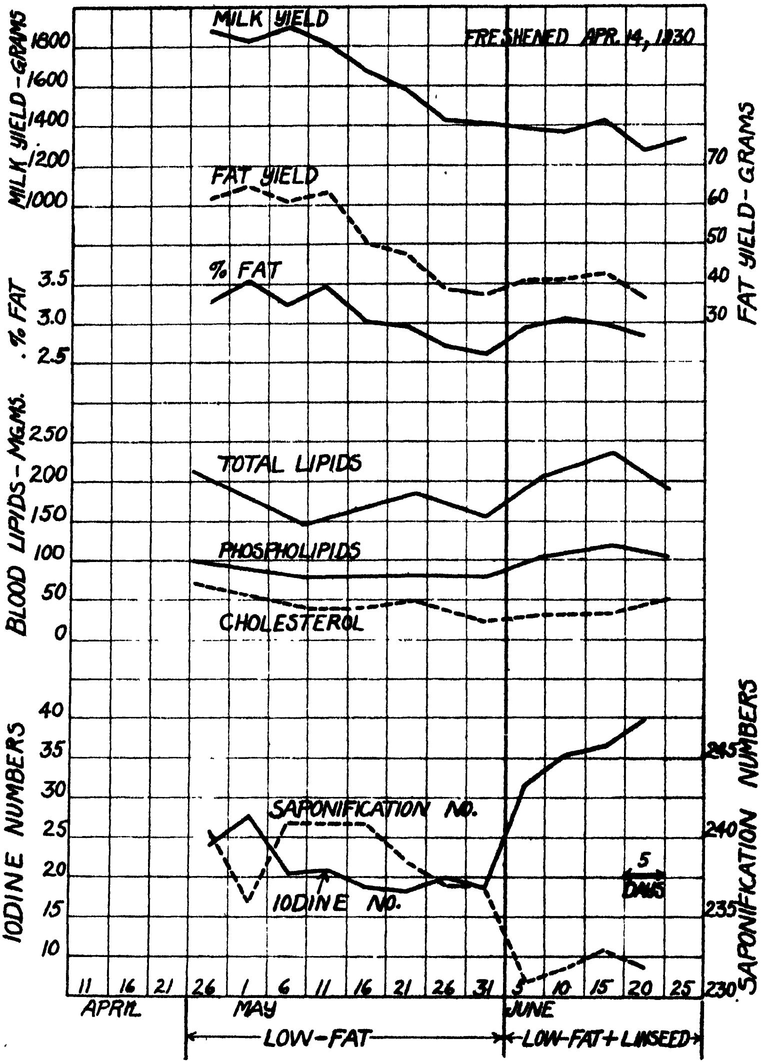

Chart V. Results Obtained with Goat D

milk yield was frequently accompanied by a tendency of the fat percentage to rise.

In the three cases where the low-fat period was followed by one in which linseed oil or cocoanut oil was fed, the large and continuous decreases mentioned above ceased and were replaced by increases in yield of milk or fat or both. While these increases are small, they represent marked changes in the trends of the curves which occurred in opposition to the normal trend of advancing lactation, and as a result of the substitution of fat for carbohydrate in a ration which supplied total digestible nutrients far in excess of the requirements. In view of these considerations the data are considered to indicate a positive effect for the oils. It is recognized that this phase of the work is unsatisfactory because of the few animals and short periods involved. It is planned to continue these studies.

In no case did the oil cause as large an increase in milk and fat yield as in the one case where the low-fat ration was replaced by the normal one. This limited observation may indicate that some other benzine-soluble constituent besides fat is involved, a question which is being further studied.

The curves for the blood lipids in the various charts reveal marked changes with the changes in ration. A significant feature shown throughout is that the curves tend to parallel each other as the values rise and fall, indicating a close metabolic relationship among these three lipids. The phospholipids, which are considered to have a special significance in fat secretion, exhibit the same changes as the others. The picture shown in chart II is similar to that found for cows (1). The various blood lipids drop during the low-fat period and rise during the following normal period, as does the milk and fat yield. In the other charts the data in general show a drop for the blood lipids during the low-fat period followed by rises with the changes to the oil rations. There is no difference between the linseed oil and the cocoanut oil as regards its influence upon the blood picture.

The striking feature of the curves for the milk fat constants is the inverse relationships shown. As the iodine number goes up the saponification does down, and *vice versa.* It is shown in chart II that the low-fat ration resulted in the secretion of a more saturated fat of a lower molecular weight than where the ration of normal fat content was employed. The same is shown in chart III, with the exception of the one set of values in the low-fat period when this goat was off-feed. Here a striking temporary change is shown where reserve fat was being drawn upon to supply the milk. Engel (6) and Henriques and Hansen (7) have shown that the iodine number of the milk fat rises during inanition and Maynard and McCay (1) report a similar observation. In our data it is shown that this is accompanied by a fall in the saponification number.

It is seen in the charts that the linseed oil resulted in a fat similar to that produced upon the normal ration. On the other hand the feeding of cocoanut oil produced no changes in either the iodine or saponification number from the values found upon the low-fat ration. These results were not unexpected.

A significant feature of the data for the fat constants is the rapidity with which the changes occur upon a change of ration. This suggests that when the ration is adequate in amount it furnishes the materials from which milk fat is made without the intervention of the fat depots.

SUMMARY

In four out of five cases, the feeding of a ration containing a superabundance of protein and total digestable nutrients, but only 0.45 per cent of ether extract, resulted in decreases in milk yield ranging from 25 to 55 per cent and in a fat yield from 35 to 70 per cent over periods from 15 to 40 days in length. In the fifth case, in which the low-fat ration was fed for 15 days shortly after freshening, no decrease in yield occurred.

The substitution for the low-fat ration of an equicaloric ration containing approximately seven per cent of ether extract caused the yield of milk and fat to rise. In three cases the substitution of linseed oil or cocoanut oil for an equicaloric amount of starch in the low-fat ration caused the downward trends in milk and fat yield to cease and small increases in either milk or fat yield resulted. In a fourth case, where the linseed oil ration followed the period of low-fat feeding in which no decreases in yield occurred, no change in milk yield resulted but the yield of the fat rose. These limited observations with the oils are interpreted to indicate that at least one of the causes of the lowered yields on a benzine-extracted ration is a lack of fat *per se,* but it is recognized that this question requires further study.

Periodic studies of the blood lipids revealed marked changes which in general followed the changes in fat intake. In all cases the curves for total lipids, phospholipids, and cholesterol exhibited a marked parallelism as the values rose and fell under the influence of the various diets. A close metabolic relationship was thus indicated.

The low-fat diet and the cocoanut oil diet resulted in the secretion of a fat which was much more saturated and lower in molecular weight than was produced on the seven-per cent fat ration or the linseed-oil ration. The curves for the iodine number and saponification number exhibited an inverse relationship throughout as the animals were shifted from one ration to another. The changes in these constants occurred sharply with the change in ration in such a manner as to indicate that, with a ration adequate in total digestible nutrients, the secretion of fat occurs with little intervention from the fat depots.

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RELATION OF TEMPERATURE OF ICE CREAM TO THE DISTRIBUTION OF CERTAIN OF ITS COMPONENTS BETWEEN THE LIQUID AND SOLID PHASES

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INTRODUCTION

The freezing of ice cream results in physical changes, most important of which, from the point of view of the texture of ice cream, is the manner in which the ice is formed.

Many discussions on "freezing ice cream" deal largely with factors affecting the whipping properties of mixes, but give little consideration to the actual freezing itself. Literally speaking, freezing does not start until ice begins to form and is not completed, providing the temperature is lowered sufficiently, until all of the free water has been changed to ice. This report is confined largely to a consideration of the relation of temperature to the distribution of various components of the mix between the liquid and solid phases. The amount of ice formed at certain temperatures and the rate at which crystallization takes place are considered in relation to their influence upon the quality of ice cream manufactured under commercial conditions. It is recognized, however, that ice cream texture is influenced by factors other than methods of freezing.

HISTORICAL

Hall (1), by observing changes in the temperature of a given amount of water due to the addition of ice cream of known temperature and weight, was able to calculate approximately the amount of ice formed at different stages in the freezing process. In addition he found, unless excessive overrun interfered, that the harder the ice cream was frozen in the freezer the smoother was the texture of the finished product.

Zoller (2) reported the use of calorimetric measurements as a means of calculating the amount of ice formed at different stages in the freezing process. Definite temperatures were not given for the stages of freezing reported. In addition he constructed a curve from calorimetric data showing the relation of temperature to the total heat capacity of ice cream.

Leighton (3), as a result of freezing point determinations, devised a means of calculating the amount of ice formed in ice cream. He assumed as a basis for calculation that ice was the only component separating out during the freezing process and that water removed as a result of evaporation under reduced pressure had the same effect upon the freezing point

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of the liquid remaining as the removal of an equal quantity of water in the form of ice.

Dahlberg (4) using the method outlined by Leighton calculated the amount of water and ice which would be present at various temperatures in ice cream containing 12 per cent fat, 10 per cent milk solids-not-fat and 14 per cent sugar.

A search of the literature has failed to reveal any report of attempts to separate the liquid phase of ice cream from its solid phase, by applying centrifugal force or by the use of a pressure filter. In this study both of these methods were employed.

METHODS

Most of the observations were made using a standard ice cream mix calculated to contain 11 per cent fat, 10.5 per cent milk solids-not-fat, 15 per cent sugar and 0.35 per cent gelatin, (Bloom Test 245).

Temperatures for both cooling and melting curves were obtained by using either a twenty-junction copper-constantan thermocouple or by the use of an accurate thermometer graduated in 0.1° C. covering the desired range. In the former case the constant temperature junction was maintained at 0° C. with a maximum variation of ± 0.01° C. as determined by a certified Beckmann thermometer. The zero current flow method of measuring E. M. F. was used in which a Leeds and Northrup type K potentiometer and sensitive galvanometer were employed for the determinations.

For the melting determinations about 40 grams of the ice cream were placed in a cylindrical copper container. Around this cylinder, but electrically insulated from it, was a coil of high resistance wire, with copper leads, which could be attached to a battery as a source of current. Current flow in some cases was applied as a source of heat thereby increasing the rate of melting. This cylinder containing the sample was then placed inside of a silvered Dewar tube and the latter in turn placed inside of a larger Dewar tube. Both tubes were stoppered but provision was made for the insertion of the thermometer or the copper leads and the thermocouple. This served as a means of obtaining the desired rates of heat absorbed by the samples.

Centrifugal force was used in the first attempts to separate the ice from the liquid portion of the ice cream. The centrifuge used was stationary, and the lack of facilities to maintain the desired temperature while centrifuging the sample made it necessary to abandon this method for one employing the principle of filtration.

A modification of the Columbia filter proved satisfactory for filtering ice cream which was not too low in temperature, but the cylinder was not constructed to withstand pressures exceeding 200 to 300 pounds per square inch.

Filtering at low temperatures required the use of pressures in excess of 500 pounds per square inch in some cases, consequently, a special cylinder was made to withstand pressures up to 1,000 pounds per square inch. One end of the filter was perforated and provision made to retain a sheet of filter paper on top of the perforations. A hydraulic press was used to apply pressure to the piston which moved in the cylinder. The capacity of the cylinder was approximately one pint. It was found that Whatman No. 40 filter paper proved satisfactory since it retained the ice crystals and allowed most of the liquid portion of ice cream to pass through.

The liquid portion passing through the filter, and the portion retained on the filter, as well as a portion of the original samples, were analyzed for fat, protein, lactose, ash and total solids. Sucrose and moisture were then calculated by difference. The fat and total solids were determined by the Mojonnier method, protein was calculated from Kjeldahl nitrogen determinations, lactose was determined by the gravimetric method using Munson and Walker tables and ash by the method described on page 260 of Methods of Analysis, A. O. A. C. (1925). Analyses were always run in duplicate and, where necessary, triplicate analyses were made to assure satisfactory checks.

EXPERIMENTAL

Cooling and melting curves were studied because it was thought that the type of curve would be an indication of the amount of ice formed when cooled over a given temperature range. It is a demonstrated fact that for every pound of ice formed under standard conditions approximately 144 B. T. U. are liberated, the exact amount depending upon the temperature at which the ice is formed. No standardized calorimeter was available, consequently, the results of these observations cannot be used for calculating quantitatively the amount of ice formed at any one temperature.

Standardizing the Thermocouple—The thermocouple used was standardized and figure 1 represents the relation between the temperature expressed in °C. and the E. M. F. of the thermocouple, expressed in microvolts. It will be noted that it is almost a straight line relationship. This chart was used to convert the E. M. F. reading of the thermocouple through the potentiometer to °C. in obtaining the data for the cooling and melting curves.

One of the melting curves is reproduced in figure 2 as an example of the type of curves obtained. Since the rate of heat absorption was nearly constant over the total range, the time required to effect one degree rise in temperature at one part of the curve as compared to the time required to obtain the same temperature rise at another part of the curve was an indication of the relative heat capacities of the ice cream at the temperatures in question. This in turn indicated the relative amounts of ice formed and was reflected in the slope of the curve.

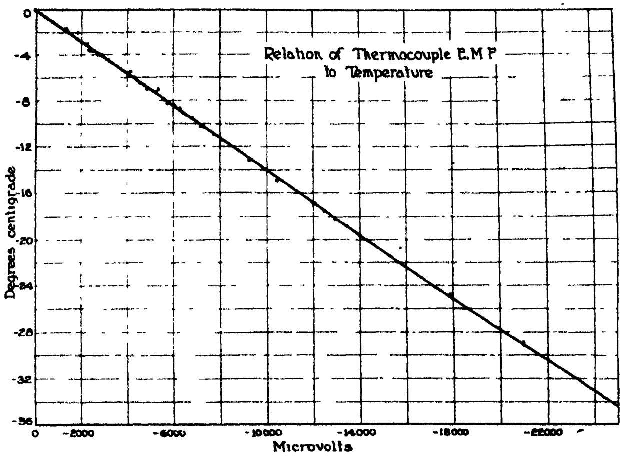

FIG. 1

The filtering process was used over a temperature range from –11.1° C. (12° F.) to –3.3° C. (26° F.). The time required to filter varied from a few minutes at the higher temperatures to several hours at the lower tem-

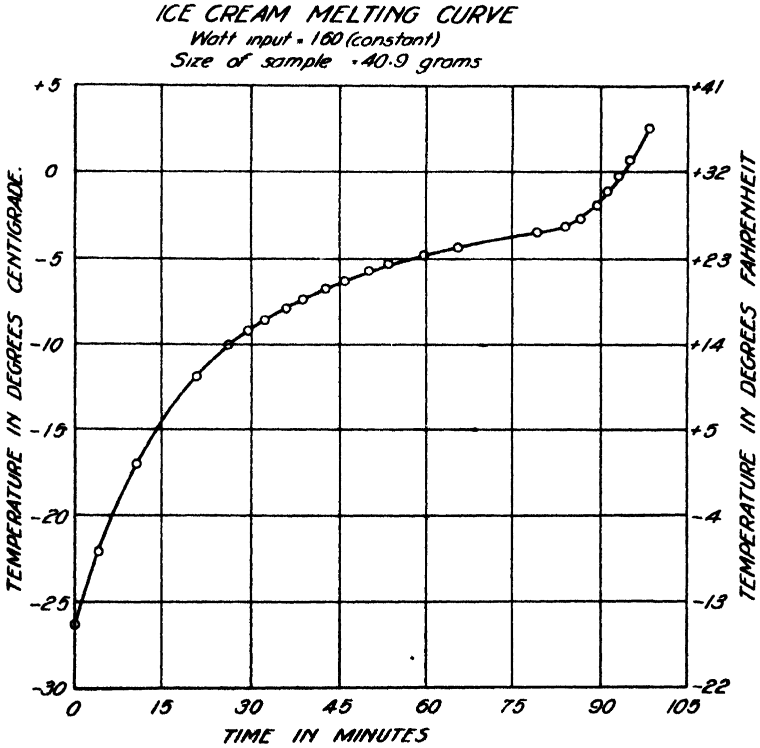

FIG. 2

peratures. The residue in each case was a fairly solid mass at the filtering temperature, but at room temperature resembled whole milk or milk which had been watered. The filtrate on the other hand was fluid at the filtering temperature. At the lower temperatures it was very viscous, and flowed only slowly.

The data obtained from the chemical analysis of the samples of filtrate and residue were used in two considerations: (1) As a basis for determining whether or not differences in temperature during the freezing process influence the amount of the components other than moisture which appear in the solid phase. (2) As a basis of estimating the relation of temperature to the amount of ice which separates during freezing.

Filtering Ice Cream—Much more satisfactory results were obtained with the filter than with the centrifuge. The temperature could be controlled and the separation of the ice was more complete in the former.

It was not possible, however, to obtain a filter which would be selective for ice, lactose, fat or any other component of the mix. Selection was confined to choosing either a fine, medium or coarse filter. Obviously this selection was arbitrary, since filters are available with openings ranging in size from those which will retain molecularly dispersed sucrose to coarse filters used for qualitative analysis. In addition, filter cloths and other coarse filters are used for some purposes.

It was decided that a quantitative filter paper would be satisfactory. This would not retain substances in the colloidal realm but would prevent ice from passing through.

PROPORTION OF VARIOUS COMPONENTS PASSING THROUGH FILTER

Table 1 gives the percentages of the various components which passed through the filter at different temperatures. It will be noted that at the

TABLE 1

*Percentages of the various components of ice cream present in filtrates at different temperatures**

SAMPLE NUMBER		91	93	95	97
FILTERING TEMPERATURE		-3.33° C. (26.0° F.)	-4.55° C. (23.8° F.)	-6.78° C. (19.8° F.)	-11.11° C. (12.0° F.)
FRACTION OF THE VARIOUS COMPONENTS RECOVERED IN THE FILTRATE	Fat	98.7	93.5	89.4	71.9
	Protein	99.0	94.9	88.9	70.3
	Lactose	97.6	93.3	89.7	69.8
	Sucrose	96.9	94.8	90.4	71.7
	Total solids	96.7	94.6	89.2	71.6
	Water	82.7	57.3	42.5	23.0

* The freezing point of the mix used in this series was -2.58° C. (27.4° F.).

lower temperatures less of each of the components passed through the filter, but that the percentages of fat, protein, lactose, sucrose, and total solids passing through at any one temperature were practically the same. The percentage of the original moisture, however, which appeared in the filtrate at each of these temperatures was very different from the percentages of other components.

Because of the nature of the various components present in ice cream it cannot be assumed that all of the residue at the various temperatures represents the solid phase. The sucrose is almost certain to be in solution and since the percentages of the components other than moisture present in the original sample which appear in the filtrate at each of the temperatures are practically the same as the percentage of the sucrose, it seems safe to assume that the lower temperatures have not caused any of the components except water to crystallize out.

In a normal ice cream mix butterfat has already solidified before freezing begins, but it is so finely dispersed that it takes on certain colloidal properties. No attempt was made to determine what changes took place in the proteins of the mix as a result of the low temperatures. Lactose in the mix was in sufficient concentration to be super-saturated at the low temperatures. Had crystallization taken place, however, part of the lactose would have been retained on the filter and there would have been a difference in the percentage of lactose passing through the filter at various temperatures as compared with the percentage of other components appearing in the filtrate. Lactose crystallization can be expected to occur over the temperature range studied providing sufficient time is allowed for equilibrium to be established.

In this comparison the time between drawing the ice cream from the freezer and the completion of filtering varied from a few hours at the higher temperatures to a maximum of two days at the lower temperatures. It would seem that the interval of time was not sufficient to allow for lactose crystallization to occur over this temperature range with the concentrations employed.

FRACTION OF THE ORIGINAL SAMPLE RETAINED AS RESIDUE

Data on the weights of the residues at different temperatures compared with the weights of the original samples are reported in table 2. As was expected, greater percentages of the sample were retained as residues when lower temperatures of filtering were employed. Curve A of figure 3 also represents, for one sample, the relation of temperature to the percentage of the sample retained as residue.

AMOUNT OF ICE FORMED AT DIFFERENT TEMPERATURES

It was apparent from the appearance of the residues that all of the liquid had not passed through the filter, and that because of the difficulties

TABLE 2

Effect of Temperature upon Amount of Residue and upon Ratio of Residue to Filtrate

MIX FROM WHICH SAMPLES WERE OBTAINED	TEMPERATURE °C.	GRAMS OF FILTRATE PER 100 GRAMS OF SAMPLE	GRAMS OF RESIDUE PER 100 GRAMS OF SAMPLE	RATIO OF RESIDUE TO FILTRATE
A	- 3.88	79.00	21.00	0.27
A	- 5.45	70.90	29.10	0.41
B	- 3.78	79.10	20.90	0.26
B	- 5.58	71.25	28.75	0.40
C	- 3.33	88.62	11.38	0.13
C	- 4.55	70.85	29.15	0.41
C	- 6.78	59.42	40.58	0.68
C	-11.11	40.75	59.25	1.45

of filtering proportionately more of it was retained at the lower temperatures.

For reasons already indicated, it seems safe to assume that the sucrose in the residue was held there in aqueous solution. It is also reasonable to assume that the percentage concentration of sucrose in aqueous solution in the residue is the same as the percentage concentration of sucrose in aqueous solution in the filtrate. Working on this assumption and knowing the amount of sucrose in the residue and the percentage sucrose in aqueous solution in the filtrate it was possible to calculate the amount of free moisture in the residue. Subtracting the sum of the weights of free moisture thus calculated and of the dry matter in the residue, from the weight of the residue at any one temperature gives a value which in this work has been considered the weight of ice formed at that temperature.

The amount of ice formed at different temperatures in one of the series of comparisons, expressed in per cent of original sample, also as per cent of the moisture in the original sample, is represented graphically in figure 3.

It is very apparent that the amount of ice formed per degree drop in temperature is much greater in the temperature range just below the freezing point of the mix than is the case at a lower temperature range.

THE EFFECT OF USING PRESSURE IN FILTERING ICE CREAM

A consideration of the phase diagram for water at high pressures shows that its freezing point is a function of pressure. The pressure used in this study varied from pressures slightly above atmospheric to 500 pounds per square inch (34 atmospheres). These pressures are relatively low compared to those required to appreciably change the freezing point of water,

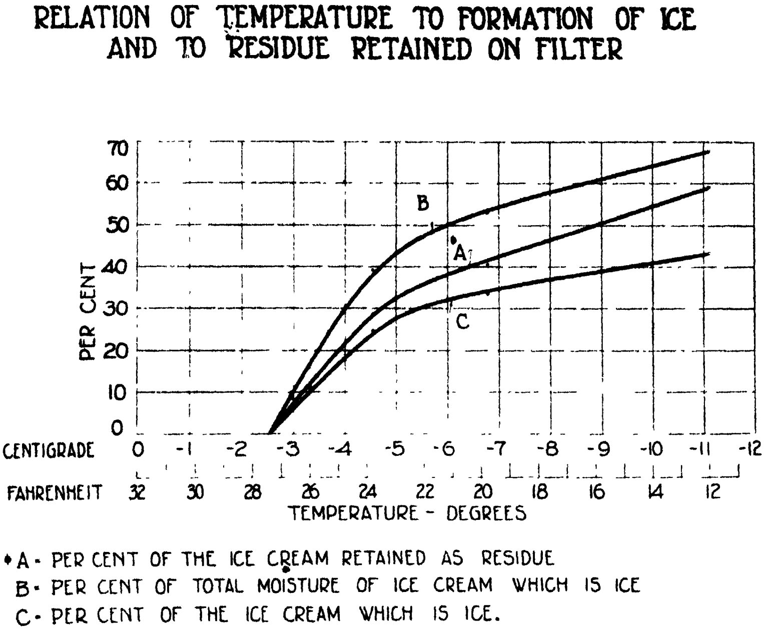

FIG. 3

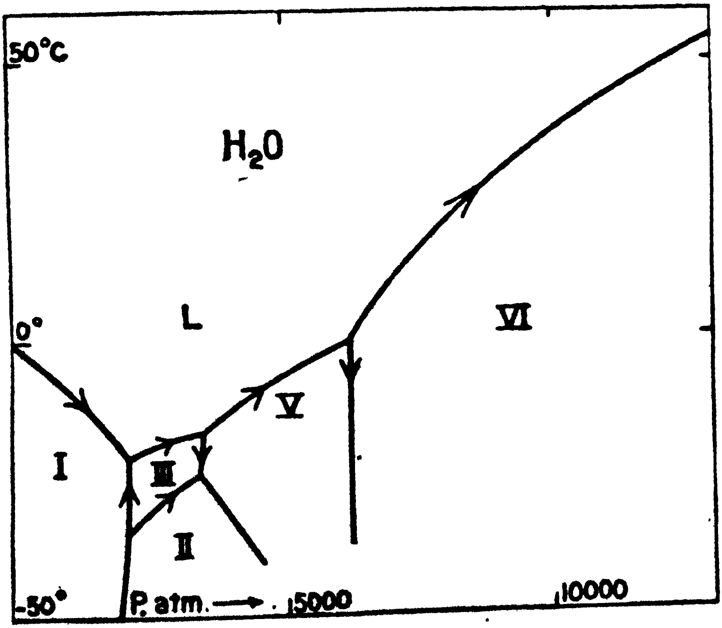

FIG. 4
Equilibrium in the system: Water and ice

as can be seen from figure 4 (5). This was verified for the case at hand by making a series of comparisons, in which the pressures applied in filtering were varied from 75 pounds per square inch for 5 minutes to 300 pounds per square inch for 5 hours.

The results of one of these comparisons where the temperature of observation was – 5° C. (23° F.) are given in table 3. The weight of the sample placed in the filter at the start was 213 grams and contained 12.19 grams of lactose. To start with, the liquid portion of the mix would be uniform in its composition with respect to lactose. Hence, any decrease in the percentage of lactose in the successive filtrates would be due to dilution of the sample as a result of ice melted. Since the filtrates and residue were analyzed for lactose, there were sufficient data available to calculate the amount of ice melted due to the filtering pressure.

TABLE 3

Relation of Pressure Applied to the Amount of Ice Melted

SAMPLE		*PRESSURE APPLIED		FRACTION OF THE ORIGINAL SAMPLE %	LACTOSE IN SAMPLE %	WT. OF SAMPLE IF NO DILUTION HAD OCCURRED GRAMS	WT. OF ICE MELTED AS RESULT OF PRESSURES APPLIED GRAMS
		Lbs. /sq. inch	Time applied				
Filtrate	1st	75	5 minutes	55.08	8.32	117.30	0.00
	2nd	75	1 hour	7.14	8.27	15.10	0.10
	3rd	250	1 "	1.97	8.02	4.08	0.12
	4th	300	5 hours	3.42	7.21	6.32	0.98
**Residue				32.39	0.457	70.2	1.20
TOTALS		75 – 250 – 300 –	1 hr.–5 min. 1 hr. 5 hrs.	100.0		213.0	1.20

* The pressure applied to the residue in obtaining any sample was the pressure indicated for that sample plus the total pressure which had already been applied in obtaining the previous sample, *e.g.*, in obtaining the 3rd filtrate the pressure applied to the residue was: 250 lbs./sq. inch for 1 hr. plus 75 lbs./sq. inch for 1 hr. and 5 min.

** The residue was that portion of the original sample remaining after the four filtrates had been obtained.

In collecting the samples used to secure the data for the amount of ice formed at various temperatures, the filtrate obtained at a given temperature included all of the filtrate from that sample. Hence, any dilution which might occur due to the melting of ice as a result of pressure applied would be very slight. If all of the lactose present in the filtrates obtained in the above series is considered present in one filtrate sample, it would test 8.25 per cent instead of the tests indicated. This is only 0.07 per cent less than the highest test obtained in the series, and since the regular method of sampling used would not allow for a dilution greater than that, the filtrate obtained could vary in composition between 8.32 per cent and 8.25 per cent lactose.

From the data presented graphically this sample would have approximately 43 per cent of the water frozen at – 5.0° C. (23° F.). This is equivalent to 57.7 grams of ice in the sample under consideration. Since it has been shown that 1.20 grams of the ice were melted due to the pressure applied, the amount of the ice formed and subsequently melted would be 2.1 per cent. Normally the sample would have been collected not later than the third filtrate, in which case the error due to dilution would be only 0.21 per cent, since most of the melting occurred during the last 5 hours in the press.

Although somewhat higher pressures were used for filtering samples at temperatures below – 5° C. (23.0° F.), it is true that at the lower temperatures the tendency for the samples to melt at a given pressure would be less. It seems, therefore, that the melting of the ice in the samples as a result of the pressures applied in these observations is a minor source of error.

DISCUSSION

The complexity of ice cream, both chemically and physically, makes difficult a study of the relationship of temperature to the changes which occur in such a mixture.

All of the methods so far used to determine the amount of ice formed as a function of temperature are indirect methods. Certain assumptions are made as a basis of calculation. This applies equally well to the filtering process used here, although it seems that the method is somewhat more direct than the calorimetric method, due to the fact that the specific heats of the various components over the temperature range studied are not definitely known.

It is recognized that the freezing point of the mixture is a function of pressure, and that excessive pressures for long periods of time will result in the melting of a portion of the ice formed at that temperature prior to the application of the pressure. It has been shown, however, that the extent of the pressure and the time it was applied in the filtering method used in this study are not sufficient to cause any significant decrease in the amount of ice retained in the residue.

It would be more desirable if the filtering method used would permit of the complete separation of the ice from the remainder of the components of the mix. Since this was not the case, the weight of the residue could not be regarded as the weight of the ice in the sample. It was possible, however, from the data available, to calculate the percentage of the residue which was ice at the temperature of observation; hence, the weight of the ice in the sample could be determined.

It is apparent that the rate of freezing in the ice cream freezer is much more rapid than subsequent freezing in the hardening room. Where ice cream is drawn from the "freezer" at low temperatures, a large portion of the ice is frozen rapidly. Consequently, a large number of small ice crystals should be formed and the resulting ice cream would be expected to have a fine smooth texture. Where the temperature of drawing from the "freezer" is relatively high, however, only a small portion of the ice is frozen rapidly. Hence, more of the ice frozen would be in the form of large crystals and the texture of the resulting ice cream would be coarser.

In the event that the rate of freezing subsequent to drawing the ice cream from the "freezer" were the same as the rate of freezing while in the "freezer," it appears that there would be no advantage, from the point of view of improving the texture, in freezing a large portion of the ice in the "freezer." In this case the principle object of putting it in the "freezer" would be to incorporate the desired amount of air. At present, however, under practically all commercial methods of ice cream manufacture, there is a marked difference in the rate of freezing in the "hardening room" as compared to the rate in the "freezer"; consequently, the temperature of "drawing" should be considered important in determining the texture of the finished ice cream (6). It should be apparent also that the advantages of rapid freezing are lost, if enough of the ice formed in the "freezer" is allowed to melt before continuing the freezing process in the hardening room. Thus, placing ice cream in warm containers or allowing it to stand at warm temperatures are conducive towards coarse textured ice cream. Fluctuating temperatures sometimes referred to as "heat shocks" are conducive to the growth of ice crystals. On the other hand, low storage temperatures maintained at a uniform level tend to retard the growth of these crystals once they are formed.

The application of the principle of rapid freezing to commercial ice cream manufacture is gaining ground. Low temperature refrigerants, high speed dashers, proper alignment and sharpness of scraper blades, as well as modifications in the methods of hardening ice cream, have been used as a means of increasing the rate of freezing. It is expected that these improvements will be accompanied by further industrial developments which will make possible still greater advantages as a result of the application of the principles of rapid freezing in ice cream manufacture.

SUMMARY AND CONCLUSIONS

1. Changes which occur in the freezing of ice cream have been shown to be chiefly concerned with the distribution of moisture between the liquid and solid phases.

2. A study of cooling and melting curves of ice cream indicated the temperature range at which maximum ice crystallization occurred to be just below the freezing point of the mix.

3. Quantitative data substantiating these observations were obtained as a result of the application of a filtering process to ice cream over a range of temperatures.

4. The significance of these observations was considered in relation to the influence of methods of freezing upon the texture of ice cream manufactured under commercial conditions, and an explanation is given for the improvement of the texture of ice cream accompanying low temperatures of drawing from the "freezer."

ACKNOWLEDGMENTS

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The author also gladly acknowledges the helpful suggestions of G. A. Richardson, Dairy Industry Division, University of California, in the preparation of this manuscript.

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CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS.
V.—THE RELATIVE ASSIMILATION OF CALCIUM FROM GRADES 1 AND 3 TIMOTHY HAY

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INTRODUCTION

Differences in the assimilation by dairy cows of calcium from alfalfa hay cured by different procedures, were first shown by Hart and his collaborators in Wisconsin. They found that hay that had been "cured under caps" gave a better assimilation than that which had been "exposed in windrows to air and light for four days" (1, 2).

The fact that the calcium of well-cured alfalfa hay is better assimilated by dairy cows than that of poorly-cured alfalfa hay was demonstrated in work conducted at this station (3). Alfalfa was cut and, after a few hours' drying in the field, spread loosely over racks in an open shed where it was exposed to the air but to no sun or rain. Another lot of the same hay was left in the field for more than a week where it was exposed to the sun and to one and one-half days of rainy weather. When these hays were fed the retention of calcium from the weathered hay was 14–15 grams per day less than that from the hay cured under cover. Assuming that the lesser calcium retention with the alfalfa exposed to sun and rain was probably due to a vitamin D deficiency,[1] an attempt was made to remedy this by the administration of cod-liver oil for a period of three weeks. No improvement in calcium assimilation was noted. In fact there was a greater loss of calcium from the body when the cod-liver oil was fed.

The question as to the effect of curing on the quality of hay is one of considerable practical as well as scientific interest. It was therefore decided to investigate different grades of other hays and for this purpose grades 1 and 3 timothy hay were first chosen for comparison and a series of balance experiments initiated. In the first experiment, conducted during the winter months of 1929, conditions were not entirely satisfactory and the results were rather inconclusive. The experiment was, therefore, repeated with some variations in the spring of 1930.

EXPERIMENTAL

For the 1929 experiment two pure-bred Holstein cows were used. Cow 200, 10 years old, had calved three months previous to the experiment and

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[1] Steenbock *et al* later showed with rats that clover hay exposed to the sun contained more Vitamin D than that which was not so exposed (4).

was giving 13 kgs. of milk daily. Cow 208, 5 years old, had calved two and one-half months previous to the experiment and was giving 8.5 kgs. of milk daily. Cow 200 maintained her weight through the experiment, while cow 208 gained 30 kgs. Both cows received timothy hay for eight months previous to the experiment, it being of No. 1 grade during the last month.

A ration of U. S. Grade 1 timothy hay and a grain mixture of corn meal, 30; linseed oil meal, 25; soy-bean meal, 25; wheat bran, 20; and salt, 1, was fed during the first four weeks of the experiment. During the next five weeks U. S. Grade 3 timothy hay, with the same grain mixture, was fed. The same ration was fed during the final three weeks of the experiment and a supplement of 50 grams per day of sodium citrate was included. A sup-

TABLE 1

Percentage composition of feeds

PERIOD	FEED	H_2O	Ca	P	N
Jan. 3–30	Timothy Hay, T 16 Grade 1	11.41	0.175	0.120	0.892
Jan. 31–Feb. 22	Timothy Hay, T 13 (1) Grade 3	10.80	0.240	0.103	0.656
Feb. 23–March 6	" " " (2)	10.57	0.235	0.101	0.644
March 7–27	" " " (3)	10.04	0.240	0.102	0.653
Jan. 3–15	Grain 65 M 1	11.74	0.686	0.620	4.00
Jan. 16–29	" 65 M 2	11.42	0.657	0.626	3.96
Jan. 30–Feb. 12	" 65 M 3	11.45	0.659	0.631	3.96
Feb. 13–28	" 65 M 4	11.78	0.774	0.777	3.90
March 1–6	" 65 M 5	12.56	0.687	0.758	4.04
March 7–23	" 65 M 6	11.70	0.747	0.783	3.98
March 24–27	" 65 M 7	12.01	0.739	0.711	3.87

TABLE 2

Percentage composition of milks

1929	COW 200			COW 208		
Week	Ca	P	N	Ca	P	N
Jan. 5–11	0.110	0.110	0.508	0.101	0.087	0.507
" 12–18	0.110	0.106	0.472	0.103	0.084	0.481
" 19–25	0.113	0.106	0.501	0.103	0.088	0.502
" 26–Feb. 1	0.111	0.108	0.502	0.099	0.089	0.507
Feb. 2–8	0.115	0.108	0.515	0.102	0.088	0.489
" 9–15	0.111	0.111	0.534	0.096	0.088	0.500
" 16–22	0.107	0.109	0.510	0.099	0.083	0.487
" 23–Mar. 1	0.108	0.110	0.521	0.095	0.087	0.495
Mar. 2–8	0.118	0.111	0.526	0.101	0.086	0.496
" 9–15	0.112	0.112	0.541	0.094	0.088	0.507
" 16–22	0.118	0.110	0.538	0.099	0.088	0.494
" 23–29	0.117	0.108	0.537	0.101	0.086	0.502

TABLE 3

Weekly calcium balances

Week	cow 200					cow 208				
	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield
1929	*gm.*	*gm.*	*gm.*	*gm.*	*kg.*	*gm.*	*gm.*	*gm.*	*gm.*	*kg*
Grade 1 Timothy Hay + $CaCl_2$ and limestone										
Jan. 5–11	275.7	107.8	421.9	+38.4	98.0	314.0	61.0	421.9	+46.9	60.4
Jan. 12–18	281.7	102.5	395.9	+11.7	93.2	318.2	61.6	395.9	+16.1	59.8
Jan. 19–25	291.0	105.4	407.7	+11.3	93.3	309.1	63.7	407.7	+34.9	61.8
Jan. 26–Feb. 1	277.4	100.8	407.9	+29.7	90.8	302.7	54.9	407.9	+50.3	55.5
Average	281.5	104.1	408.4	+22.8	93.8	311.0	60.3	408.4	+37.1	59.4
Grade 3 Timothy Hay + $CaCl_2$ and limestone										
Feb. 2–8	323.4	101.3	423.7	– 1.0	88.1	330.3	57.5	440.5	+52.7	56.4
Feb. 9–15	297.1	100.0	435.8	+38.7	90.1	365.2	51.3	452.6	+36.1	53.4
Feb. 16–22	314.8	89.5	425.9	+21.6	83.6	316.5	51.6	442.7	+74.6	52.1
Feb. 23–Mar. 1	238.7	86.4	417.2	+92.1	80.0	303.7	47.7	440.8	+89.4	50.2
Mar. 2–8	257.4	93.8	374.3	+23.1	79.5	310.0	49.8	403.7	+43.9	49.3
Average	286.3	94.2	415.4	+34.9	84.3	325.1	51.6	436.1	+59.3	52.3
Grade 3 Timothy Hay + $CaCl_2$, limestone and sodium citrate										
Mar. 9–15	301.1	86.9	440.7	+52.7	77.6	312.8	45.4	457.5	+99.3	48.3
Mar. 16–22	296.6	95.1	452.4	+60.7	80.6	372.2	49.2	483.6	+62.2	49.7
Mar. 23–29	283.3	88.5	424.1	+52.3	75.6	383.4	51.6	481.1	+46.1	51.1
Average	293.7	90.2	439.1	+55.2	77.9	359.1	48.7	474.1	+69.2	49.7

TABLE 4

Weekly phosphorus balances

Week	cow 200					cow 208				
	P in urine and feces	P in milk	P in feed	P balance	Ca/P ratio	P in urine and feces	P in milk	P in feed	P balance	Ca/P ratio
1929	*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm*	
Grade 1 Timothy Hay + $CaCl_2$ and limestone										
Jan. 5–11	225.1	107.8	362.6	+29.7	1.16	278.0	52.5	362.6	+32.1	1.16
Jan. 12–18	245.1	98.8	341.3	− 2.6	1.16	288.0	50.2	341.3	+ 3.1	1.16
Jan. 19–25	256.5	98.9	365.5	+10.1	1.11	269.0	54.4	365.5	+42.1	1.11
Jan. 26–Feb. 1	268.0	98.1	366.1	0.0	1.11	313.8	49.4	366.1	+ 2.9	1.11
Average	248.7	100.9	358.9	+ 9.3	1.14	287.2	51.6	358.9	+20.1	1.14
Grade 3 Timothy Hay + $CaCl_2$ and limestone										
Feb. 2–8	249.2	95.1	352.5	+ 8.2	1.20	285.4	49.6	359.7	+24.7	1.22
Feb. 9–15	279.3	100.0	368.0	−11.3	1.18	340.4	47.0	375.2	−12.2	1.21
Feb. 16–22	297.5	91.1	369.6	−19.0	1.15	340.8	43.2	376.8	− 7.2	1.17
Feb. 23–Mar. 1	289.0	88.0	365.9	−11.1	1.14	327.1	43.7	376.0	+ 5.2	1.17
Mar. 2–8	305.0	88.2	373.1	−20.1	1.00	367.1	42.4	385.7	−23.8	1.05
Average	284.0	92.5	365.8	−10.7	1.13	332.2	45.2	374.7	− 2.7	1.16
Grade 3 Timothy Hay + $CaCl_2$, limestone and sodium citrate										
Mar. 9–15	304.0	86.9	399.1	+ 8.2	1.11	346.9	42.5	406.3	+16.9	1.13
Mar. 16–22	311.4	88.7	420.4	+20.3	1.07	372.2	43.7	433.7	+17.8	1.11
Mar. 23–29	280.0	81.6	372.8	+11.2	1.14	356.2	43.9	411.0	+10.9	1.17
Average	298.5	85.7	397.4	+13.2	1.11	358.4	43.4	417.0	+15.2	1.14

TABLE 5

Percentage composition of feeds

PERIOD	FEED	H_2O	Ca	P	N
March 13–April 7	Timothy Hay, T 22 (1) Grade 3	7.83	0.393	0.124	0.694
April 8–May 9	" " " (2)	11.27	0.367	0.129	0.611
May 10–June 4	" " " (3)	10.76	0.391	0.126	0.594
June 5–27	Timothy Hay, T 21 Grade 1	8.70	0.397	0.114	0.971
March 13–20	Grain 65 M 1	10.95	0.616	0.668	3.90
" 21–29	" 65 M 2	10.87	0.730	0.736	4.10
" 30–April 2	" 65 M 3	10.91	0.729	0.723	4.10
April 3–11	" 65 M 4	10.92	0.657	0.718	4.17
" 12–20	" 65 M 5	12.00	0.619	0.724	4.06
" 21–23	" 65 M 6	10.90	0.634	0.743	4.10
" 24–May 3	" 65 M 7	10.99	0.735	0.741	4.11
May 4–11	" 65 M 8	9.96	0.738	0.736	4.10
" 12–14	" 65 M 9	10.60	0.754	0.758	4.23
" 15–25	" 65–1	10.41	0.233	0.777	4.29
" 26–June 3	" 65–2	10.73	0.206	0.722	4.16
June 4–13	" 65–3	10.46	0.210	0.732	4.41
" 14–23	" 65–4	11.93	0.190	0.707	4.32
" 24–27	" 65–5	9.71	0.215	0.697	4.29

TABLE 6

Percentage composition of milks

1930	COW 266			COW 270		
Week	Ca	P	N	Ca	P	N
March 15–21	0.106	0.096	0.481	0.121	0.106	0.508
" 22–28	0.111	0.099	0.523	0.115	0.107	0.529
" 29–April 4	0.110	0.096	0.519	0.119	0.106	0.527
April 5–11	0.110	0.097	0.564	0.119	0.107	0.535
		COW 105				
" 12–18	0.098	0.095	0.481	0.118	0.108	0.533
" 19–25	0.104	0.099	0.497	0.128	0.108	0.538
" 26–May 2	0.097	0.097	0.497	0.118	0.104	0.528
May 3–9	0.099	0.091	0.482	0.123	0.106	0.522
" 10–16	0.098	0.094	0.490	0.115	0.110	0.531
" 17–23	0.096	0.095	0.496	0.119	0.111	0.546
" 24–30	0.102	0.098	0.514	0.124	0.112	0.539
" 31–June 6	0.102	0.095	0.513	0.118	0.107	0.539
June 7–13	0.102	0.099	0.515	0.116	0.111	0.547
" 14–20	0.107	0.098	0.513	0.123	0.108	0.544
" 21–27	0.105	0.101	0.524	0.128	0.108	0.546

TABLE 7

Weekly calcium balances

Week	cow 266					cow 270				
	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield
1930	*gm.*	*gm.*	*gm.*	*gm.*	*kg.*	*gm.*	*gm.*	*gm.*	*gm.*	*kg.*
Grade 3 timothy hay + $CaCl_2$, limestone and sodium citrate										
Mar. 15–21	575.0	188.2	755.3	− 7.9	177.5	685.1	261.2	847.5	−98.8	215.9
Mar. 22–28	657.8	196.1	755.1	−98.8	176.7	720.6	247.9	929.6	−38.9	215.6
Mar. 29–Apr. 4	660.5	165.2	766.1	−59.6	150.2	747.0	253.0	942.8	−57.2	212.6
Average	631.1	183.2	758.8	−55.4	168.1	717.6	254.0	906.6	−65.0	214.7
Grade 3 timothy hay + $CaCl_2$ and limestone										
Apr. 5–11	453.6*	116.3*	511.2*	−58.7*	105.7*	680.4	257.9	875.8	−62.5	216.7
	cow 105									
Apr. 12–18	481.0	144.3	573.4	−51.9	147.2	600.2	253.1	837.4	−15.9	214.5
Apr. 19–25	470.8	153.5	573.8	−50.5	147.6	672.4	273.9	833.7	−112.6	214.0
Average	475.9**	148.9**	573.6**	−51.2**	147.4**	651.0	261.6	849.0	−63.7	215.1
Grade 3 timothy hay + limestone										
Apr. 26–May 2	540.5	145.7	642.9	−43.3	150.2	741.5	251.5	925.7	−67.3	213.1
May 3–9	517.2	149.1	644.1	−22.2	150.6	735.7	258.8	927.3	−67.2	210.4
May 10–16	528.8	143.7	657.5	−15.0	146.6	722.4	240.2	948.4	−14.2	208.9
Average	528.8	146.2	648.2	−26.8	149.1	733.2	250.2	933.8	−49.6	210.8
Grade 3 timothy hay										
May 17–23	294.7	139.6	350.1	−84.2	145.4	380.1	241.7	524.1	−97.7	203.1
May 24–30	321.3	141.8	358.5	−104.6	139.0	342.4	257.3	514.4	−85.3	207.5
May 31–June 6	267.5	141.1	349.1	−59.5	138.3	339.7	235.2	501.9	−73.0	199.3
Average	294.5	140.8	352.6	−82.8	140.9	354.1	244.7	513.5	−85.3	203.3
Grade 1 timothy hay										
June 7–13	251.0	148.0	354.6	−44.4	145.1	313.9	235.5	509.9	−39.5	203.0
June 14–20	247.9	153.1	344.7	−56.3	143.1	334.1	248.2	496.7	−85.6	201.8
June 21–27	273.3	151.3	347.6	−77.0	144.1	316.9	262.4	500.6	−78.7	205.0
Average	257.4	150.8	349.0	−59.2	144.1	321.6	248.7	502.4	−67.9	203.3

* 5 days. ** For cow 105 only.

TABLE 8

Weekly phosphorus balances

Week	cow 266					cow 270				
	P in urine and feces	P in milk	P in feed	P balance	Ca/P ratio	P in urine and feces	P in milk	P in feed	P balance	Ca/P ratio
1930	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	
				Grade 3 timothy hay + CaCl$_2$, limestone and sodium citrate						
Mar. 15–21	430.0	170.4	603.1	+ 2.7	1.25	451.3	227.8	665.3	− 13.8	1.27
Mar. 22–28	438.5	174.9	589.0	− 24.4	1.28	484.1	229.6	714.3	+ 0.6	1.30
Mar. 29–Apr. 4	469.5	144.2	590.6	− 23.1	1.30	503.5	225.4	716.2	− 12.7	1.32
Average	446.0	163.2	594.2	− 14.9	1.28	479.6	227.6	698.6	− 8.6	1.30
				Grade 3 timothy hay + CaCl$_2$ and limestone						
Apr. 5–11	314.0*	102.5*	416.7*	+ 0.2*	1.23*	517.3	231.9	708.5	− 40.7	1.24
		cow 105								
Apr. 12–18	385.6	139.8	509.8	− 15.6	1.12	485.6	230.6	715.1	− 1.1	1.17
Apr. 19–25	429.1	146.1	524.5	− 50.7	1.09	530.3	231.1	723.4	− 38.0	1.15
Average	407.4**	143.0**	517.2**	− 33.1**	1.11**	511.1	231.2	715.7	− 26.6	1.19
				Grade 3 timothy hay + limestone						
Apr. 26–May 2	399.2	145.7	530.0	− 14.9	1.21	524.8	221.6	730.8	− 15.6	1.27
May 3–9	357.8	137.0	528.0	+ 33.2	1.22	477.4	223.0	728.1	− 27.7	1.27
May 10–16	388.5	137.8	531.8	+ 5.5	1.24	483.5	228.7	732.8	+ 20.6	1.29
Average	381.8	140.2	529.9	+ 7.9	1.22	495.2	224.4	730.6	− 7.6	1.28
				Grade 3 timothy hay						
May 17–23	422.6	138.1	555.0	− 5.7	0.63	559.3	225.4	758.5	− 26.2	0.69
May 24–30	438.1	132.0	570.1	− 24.8	0.63	516.3	232.4	738.7	− 10.0	0.70
May 31–June 6	419.8	131.4	526.4	− 24.8	0.66	520.5	213.3	713.5	− 20.3	0.70
Average	426.8	133.8	550.5	− 18.4	0.64	532.0	223.7	736.9	− 18.8	0.70
				Grade 1 timothy hay						
June 7–13	419.8	143.7	525.0	− 38.5	0.68	504.5	225.3	710.7	− 19.1	0.72
June 14–20	383.8	140.2	512.6	− 11.4	0.67	490.0	217.9	694.2	− 13.7	0.71
June 21–27	403.7	145.5	507.0	− 42.2	0.69	463.6	221.4	686.7	+ 1.7	0.73
Average	402.4	143.1	514.9	− 30.7	0.68	486.0	221.5	697.2	− 10.4	0.72

* 5 days. ** For cow 105 only.

plement of calcium chloride and limestone was fed throughout the experiment in such amount as to maintain a Ca/P ratio of 1.0–1.2. The supplements were mixed with the grain, and hay and grain were fed twice daily. The cows were milked twice daily. Cow 200 was underfed (75% of Savage Standard) during three months following calving and cow 208 during one month following calving (89% of Savage Standard). Both cows received more than their energy requirements during the experiment (5–46% excess). They were not bred.

Table 1 shows the percentage composition of the feeds used and table 2 that of the milks. Tables 3 and 4 give the calcium and phosphorus balance data.

The experiment of the spring of 1930 was started with two pure-bred Holsteins, cows 266 and 270, of about 9 years of age. Both cows were in their third month of lactation and were giving 25–30 kgs. of milk daily. Previous to the experiment they were receiving alfalfa hay, corn silage and grain, but three days previous to the experiment the ration was changed to grade 3 timothy hay and grain with a supplement of calcium chloride, limestone and sodium citrate. This was fed for three weeks, then for the following three weeks the sodium citrate was omitted. For the next three weeks both sodium citrate and calcium chloride were omitted and during the fourth three weeks no supplement was given. During the fifth and final three weeks, grade 1 timothy hay and grain without supplement were fed.

During the fourth week of the experiment it became evident that cow 266, due to lameness, was unsuitable for the experiment. She was, therefore, discarded and a three year old grade Holstein, Cow 105, already receiving a timothy hay ration, was substituted. This cow was likewise in her third month of lactation. Cow 266 lost a little weight while on the experiment, but cows 105 and 270 both gained in weight. Hay and grain were fed twice daily and the cows were milked three times a day. Cows 105 and 266 were not bred but cow 270 was bred and was pregnant during the last month of the experiment.

Table 5 shows the percentage composition of the feeds used and table 6 that of the milks. Tables 7 and 8 give the calcium and phosphorus balance data.

DISCUSSION

The differentiation in the effect of different grades of timothy hay on the calcium and phosphorus assimilation is rather difficult and demonstrable only under the most favorable conditions. These conditions should include the use of as many animals as possible with high milk production so that the demand for minerals is acute.

In the 1929 experiment only two animals with rather low milk yields were available. In planning this experiment it was thought desirable to add a calcium supplement to overcome the low calcium intake and Ca/P ratio on a timothy hay ration and thereby eliminate those factors in estimating the relative value of the hays. The calcium balances were somewhat better with the grade 3 hay. This improvement in calcium balances with the grade 3 hay may be more than accounted for by the increased calcium intakes and the decreased milk yields at the time this hay was fed. Unfortunately the grade 3 hay had a higher calcium content than did grade 1.

Sodium citrate was introduced into the ration with the thought that an acid radicle which predominates in alfalfa (5) and which is capable of holding calcium in solution might be an aid to calcium assimilation. The improvement in balances noted, however, when the sodium citrate was fed, can be more than accounted for by increased calcium intakes and decreased milk yields.

With sufficient calcium intakes and low milk yields the calcium balances in this experiment are all positive.

The experiment of 1930 was planned somewhat differently. Two cows with considerably higher milk yields were used. A comparison of grades 1 and 3 timothy hay without any mineral supplement was made. Grade 3 hay with all the supplements was fed first, then one supplement at a time was removed until the grade 3 hay alone with grain was fed. After that the grade 1 hay was substituted.

The balances when the different supplements were used are probably not very significant. One cow had to be removed from the experiment and another substituted in her place. The balances are shown to be somewhat better after the calcium chloride and sodium citrate had been removed and only the limestone supplement was given. The intakes of calcium and phosphorus, however, were greater during the latter period.

When all supplements were removed the balances became strongly negative due to the decreased calcium intakes.

The comparison of grades 1 and 3 timothy hay is marked and probably of some significance. The calcium balances were distinctly better on the grade 1 hay. In this comparison there was little change in calcium intakes or in milk yields and the cows were giving considerable amounts of milk. There was a considerable reduction in the excretion of calcium in the feces when the grade 1 hay was fed.

All the balances in this experiment were negative due to the high milk yields and probably insufficient mineral intakes.

CONCLUSIONS

These experiments seem to indicate that the ingestion of sodium citrate has little effect on calcium and phosphorus metabolism.

Grade 1 timothy hay seems to exert a more favorable effect than does grade 3 timothy hay on calcium assimilation in heavily milking cows.

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A COMPARISON BETWEEN GROUND SOYBEANS AND LINSEED OILMEAL AS PROTEIN SUPPLEMENTS FOR GROWING DAIRY CALVES

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Experience has demonstrated that grain rations for farm animals in most instances should be supplemented with an adequate source of protein to give satisfactory and economical growth and production. Since most protein supplements are expensive, dairymen are becoming more and more interested in home grown protein feeds for dairy cattle. Such a source of protein is soybeans. Due to their high protein content, their yielding qualities and ability to grow on various types of soils, soybeans have attracted the attention of investigators and feeders as a source of protein supplement to the rations of different farm animals during various periods of development and production.

Numerous investigators (1, 2, 3, 4, 5) have found ground soybeans to be equal to linseed oilmeal as a protein supplement in the grain ration for milking cows. Likewise, experiments have shown that soybeans give satisfactory results for fattening hogs (6) and also for laying pullets (7). However, with young growing hogs and chicks, soybeans have not proven satisfactory as a protein supplement. Robinson (8) found that soybeans were not a satisfactory supplement to corn for weaning pigs which did not have access to forage and similar results were observed at Purdue (9). Experiments (10) with growing chicks indicated that optimum growth could not be secured with ground soybeans.

To the knowledge of the authors there have been no experimental results published on the value of ground soybeans as a protein supplement for growing dairy calves.

Since soybeans have become one of the chief sources of home grown proteins and due to the fact that most experimental work in studying soybeans as a feed for dairy cattle has been done with cows for milk production, it was decided to conduct some feeding trials for the purpose of determining the value of soybeans as a feed for growing dairy calves. Since linseed oilmeal has proven to be a satisfactory supplement to the grain ration of growing calves, it was decided to compare ground raw soybeans with linseed oilmeal as a standard in these trials.

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EXPERIMENTAL PROCEDURE

Two trials have been completed in which eight calves have been raised on ground raw soybeans as a protein supplement, and eight calves on linseed oilmeal as a protein supplement. The first trial began in May 1929 and was continued for a period of 360 days. The second trial began in September 1930 and continued for a period of 330 days. Eight calves, divided into two groups of four calves each were used in each trial. In the first trial each group contained two Ayrshires, one Holstein and one Jersey whose average age at the beginning was 64 days. The groups in the second trial contained two Ayrshires and two Holsteins each, whose average age at the beginning of trial was 89 days. The groups were made as uniform as possible in regard to age, size and weight.

The calves received whole milk to 90 days of age when milk feeding was discontinued for the reason that the milk might mask the true biological value of the linseed oilmeal and the ground soybeans as protein supplements to the grain rations. The milk was fed at the rate of one pound of milk for each ten pounds of live weight of the calf with a maximum amount of ten pounds of milk per calf per day.

One group of calves received a grain ration containing linseed oilmeal, and the other group received ground soybeans. On the basis of chemical analyses of the individual feeds used in the grain mixture, the rations were formulated so as to furnish approximately the same percentage of total protein and total digestible nutrients. Periodic analyses of the grain mixtures showed them to contain practically the same amounts of protein. The average protein content of the linseed oilmeal ration was 16.20 per cent and of the soybean ration 16.35 per cent. The composition of the two grain mixtures is given in table 1.

In the first trial both groups of calves received timothy hay for the first ninety days and then were fed alfalfa hay during the remainder of the trial. In the second trial the calves were fed alfalfa hay during the entire trial.

TABLE 1

Kinds and amounts of ingredients in the grain mixtures

	LINSEED OILMEAL RATION	GROUND SOYBEAN RATION
	Lbs.	Lbs.
Ground Corn	300	318
Ground Oats	300	350
Wheat Bran	300	300
Linseed Oilmeal	200	...
Ground Raw Soybeans	...	182
Salt	10	10

The calves consumed sufficient amounts of hay and grain to keep them in good growing condition. They were fed twice daily and accurate records kept of the feeds consumed by each calf. The calves were fed in dry lot and did not have access to pasture at any time during the experiment.

The calves were weighed at the beginning of the experiment and every ten days thereafter, weights being taken for three successive days and an average of the three weights used as the true weight of the individual at that time. Their heights at withers were taken at the beginning of the trial and from then on every thirty days. Careful observations were made for digestive disorders, and any other abnormal conditions of the individual calves used in these trials. A summary of the results of the two feeding trials is given in table 2 and the gain in weight per 30 day interval is given in Chart I.

TABLE 2

Feeds consumed, gains made in weight and height by the calves on the experiment

	TRIAL I 1929–30		TRIAL II 1930–31		AVERAGE TWO TRIALS	
	Linseed Oilmeal	Ground Soybeans	Linseed Oilmeal	Ground Soybeans	Linseed Oilmeal	Ground Soybeans
No. calves	4	4	4	4	4	4
Ave. age at beginning—days	64	64	89	91	76.5	77.5
No. experimental days	360	360	330	330	345	345
Milk—lbs.	1225.0	1081.5	526.50	743.50	875.75	912.50
Grain—lbs.	6092.5	5953.75	5805.50	5730.00	5949.1	5841.9
Hay—timothy—lbs.	632.25	599.50	—	—	—	—
Hay—alfalfa—lbs.	4857.00	4857.00	6080.5	6080.5	—	—
Total hay—lbs.	5489.25	5456.50	6080.00	6080.5	5785.6	5768.50
Total dry matter—lbs.	10660.4	10486.7	10879.3	10827.5	10769.85	10657.1
Total gain wt.—lbs.	1701	1637	1805	1865	1753	1751
Ave. daily gain—lbs.	1.18	1.14	1.36	1.41	1.27	1.27
Lbs. dry matter per 1 lb. gain	6.27	6.41	6.02	5.81	6.14	6.09
Total increase height (inches)	54.2	53.1	52.4	53.6	53.3	53.35
Ave. monthly increase in height (inches)	1.129	1.108	1.191	1.218	1.158	1.159

DISCUSSION

A study of the data in table 2 shows that the average daily gain and the average monthly gain in height at withers of the calves on the two feeds was approximately the same.

Although there was a difference between the average daily gain in trials 1 and 2, the significant fact about these gains is that within each trial the

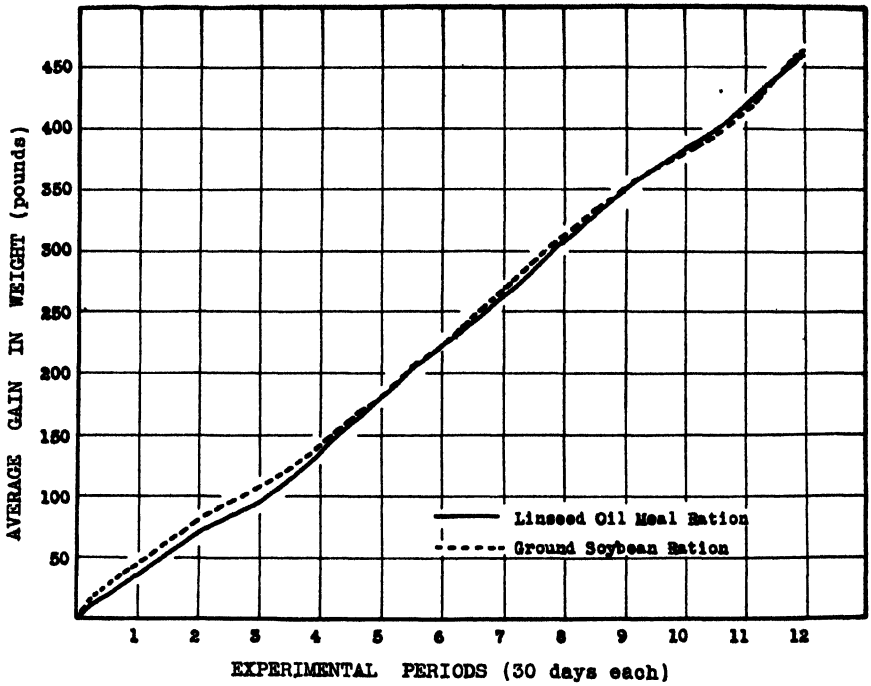

CHART I—SHOWING THE AVERAGE GAIN IN WEIGHT OF THE CALVES OF EACH GROUP DURING THE TWO TRIALS COMPARING GROUND SOYBEANS AND LINSEED OILMEAL AS PROTEIN SUPPLEMENTS IN THE GRAIN RATION.

respective groups responded almost identically to the two rations. In fact it is readily seen that the values for the various measurements of response between feed mixtures in each trial are well within the limits of experimental error. Furthermore, the similarity of response of the two groups through each period of the feeding trial is clearly shown in Chart I.

Contrary to the results obtained with hogs (7) and chicks (8), these experiments showed that soybeans can be utilized as protein supplements by calves to make satisfactory growth. All the animals in these trials maintained good health and showed no physiological disorders as evidenced by general appearance, growth and the fact that all heifers used in the first trial have passed through normal reproductive cycles.

SUMMARY

1. Two trials, 360 days and 330 days respectively, have been completed in which eight calves have been raised on alfalfa hay and a grain ration containing linseed oilmeal, and eight calves on a comparable ration in which ground raw soybeans were used in place of linseed oilmeal as a protein supplement.

2. Ground raw soybeans and linseed oilmeal were found to be equally effective as protein supplements in the grain rations for growing heifer calves when fed with alfalfa hay.

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THE EFFECT OF COD LIVER OIL IN THE RATION UPON THE QUANTITY AND QUALITY OF COW'S MILK[1]

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Until recently it has been generally believed that food exerted no marked influence upon the amount of fat in cows' milk. In recent years, however, this concept has been modified. This station, as well as others, have shown that certain food fats temporarily increased the fat content of milk. More recently there has appeared in the literature evidences that cod liver oil, menhaden oil, and safflower meal in the cows' ration has a depressing influence on the fat percentage of milk.

THE LITERATURE

Channon *et al.* (3) observed a tendency toward a decline in the fat content of cows' milk when cod liver oil was fed. This experiment was again reported by Drummond *et al.* (4) with comment to the effect that from the limited data (on three cows) they could not draw definite conclusions. From Reading, Golding and Zilva (5) and Mattick (7) reported that cod liver oil in the ration of milking cows had a depressing influence upon the fat content of the milk. Others have stated, without producing data, that cod liver oil in the ration depresses the fat content of the milk produced.

Harvey (6) found no effect on the fat content of goats' milk by feeding cod liver oil. This, coupled with the fact that the author found no depression of fat with two cows fed cod liver oil two years ago, suggested that further study of this problem was necessary.

In this connection, it is also of interest to note that Bünger (2) reports safflower meal as depressing the fat percentage of cows' milk up to 24 per cent, and Brown and Sutton (1) that menhaden (fish) oil acts similarly.

EXPERIMENTAL

The experimental animals—Twelve cows represented by Guernsey, Holstein, and Jersey breeds, and in different stages of lactation were selected. They were divided into two groups as nearly alike as possible as to breed and stage of lactation.

Feeding—The cows were fed the regular dairy herd ration consisting of alfalfa hay and corn silage for roughage while in the barn, and mixed

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pasture grasses, chiefly blue grass, when on pasture. A grain mixture of corn 1 part, barley 1 part, oats 2 parts, and cottonseed meal ½ part was fed according to production. This is hereinafter known as the basal ration. For the experimental ration, five ounces of high grade biologically tested cod liver oil was added to the basal ration daily. It was added to the grain mixture at feeding time. No difficulty was experienced in getting the cows to eat the mixture.

Plan—The experiment was divided into three periods of 15, 23, and 20 days' duration. The first served as a preliminary period, and both groups received the basal ration only. During the second period, Group I had the basal ration only, and Group II received in addition five ounces cod liver oil daily. In the third group, Group I received the oil, and Group II the basal ration only.

Testing and records—Each milking was weighed, sampled, and examined for fat content by the Babcock method. The daily milk, fat, and fat percentage records were computed for each individual and for groups by periods.

TABLE 1

The fat percentage and average daily milk production for individual cows by periods. The marked lowering of fat percentage during cod-liver oil periods is to be noted

COW NUMBER	PERIOD I		PERIOD II		DIFFERENCE PERIOD II AND I	PERIOD III		DIFFERENCE PERIOD III AND II
	FAT	MILK	FAT	MILK	FAT	FAT	MILK	FAT
	per cent	*pounds*	*per cent*	*pounds*	*per cent*	*per cent*	*pounds*	*per cent*
	Preliminary		*Basal ration*			*Cod-liver oil*		
165	4.59	38.4	4.71	35.7	+ .12	4.60	33.8	− .11
173	5.36	32.1	5.12	33.9	− .24	3.93	31.5	−1.19
383	3.95	49.7	3.33	55.0	− .62	2.46	51.0	− .87
393	3.33	67.1	3.03	64.7	− .30	2.51	61.7	− .52
402	3.61	60.1	3.20	65.7	− .41	2.13	63.1	−1.07
540	4.53	24.7	4.31	27.0	− .23	3.55	26.1	− .76
Average					− .30			− .75
	Preliminary		*Cod-liver oil*			*Basal ration*		
171	4.77	34.3	4.12	32.9	− .65	4.31	26.9	+ .19
390	3.54	60.4	2.81	51.9	− .73	2.84	53.2	+ .03
396	3.94	58.5	2.84	66.9	−1.10	2.85	64.7	+ .01
542	4.54	22.6	3.91	24.9	− .63	4.20	24.8	+ .29
549	5.97	21.2	5.34	20.1	− .63	5.93	20.6	+ .59
551	6.01	17.1	4.98	19.5	−1.03	5.26	19.8	+ .28
Average					− .63			+ .232

DISCUSSION OF RESULTS

In table 1 are given the tabulated results of the experiment. In Period II (when Group I received only the basal ration and Group II the basal ration plus cod liver oil) the average fat percentage declined for both groups. The decrease is much greater, however, for Group II than for Group I being 0.815 per cent and 0.313 per cent, respectively. For this period all cows in Group II declined in fat percentage from 0.63 per cent to 1.10 per cent, while in Group I one cow showed an increase in fat percentage, and the maximum decrease was 0.62 per cent or less than the minimum for Group II.

In Period III, when Group I received the cod liver oil ration, the fat percentage of the milk declined for every cow with an average actual decline of 0.772 per cent as compared with the preceding period. Cod liver oil was withdrawn from the ration of Group II, and an increase in the fat percentage of the milk is noted for each cow.

In figure 1, the daily average fat percentage for each group is presented graphically. It will be noticed that the depression in fat percentage begins immediately after introducing cod liver oil into the ration, and also that cod liver oil has a cumulative effect in that it continues to depress that fat content of milk. For Group I the tendency for decline in fat content continues with each day until the 17th day of feeding when the lowest point is reached. For Group II the lowest point is reached on the 19th day of cod liver oil feeding.

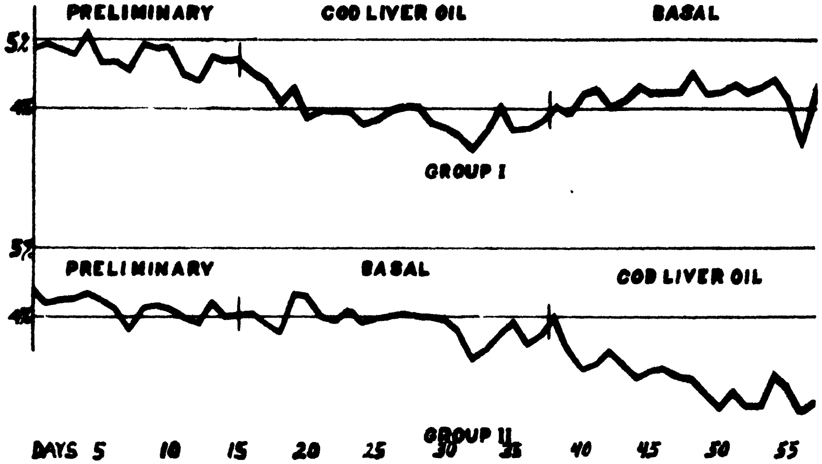

FIG. 1. COMPARISON OF DAILY AVERAGE FAT PERCENTAGE OF THE TWO GROUPS BY PERIODS. DOWNWARD TRENDS OF THE CURVE ARE NOTED WHEN COD-LIVER OIL IS INTRODUCED, AND SLOW RECOVERY TO NORMAL WHEN THE COD-LIVER OIL IS REMOVED

From figure 1, it will also be seen that the recovery to normal fat percentage is slow after removal of the cod liver oil from the ration. It will be noticed that there is a fairly constant increase in fat percentage up to the 16th day after removal of the cod liver oil.

From the data there is no evidence that cod liver oil influenced the amount of milk. The fluctuations in daily milk production appear about equal in cod liver oil periods and basal ration periods.

Summary and Conclusions—The effect of feeding cod liver oil to milking cows is reported.

In every case when cod liver oil to the extent of five ounces daily was added to the ration, there was a marked decline in the fat percentage of the milk with no apparent effect upon the amount of milk.

The depressing effect of cod liver oil seems to be cumulative for a time, at least.

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THE USE OF SKIMMILK POWDER IN GRAIN RATIONS FOR DAIRY CALVES*

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INTRODUCTION

Experimental results have shown that powdered skimmilk fed dry to calves in their grain ration is the cheapest and most satisfactory method of feeding it if milk feeding is to be continued for a period of more than two months. (1), (2), (3), (4), (5).

Recent investigational work in calf feeding is on the preparation of home-mixed calf rations to be used dry as grain rations or as calf meal gruels as substitutes for milk. Most of these mixtures contain skimmilk powder, varying in amount from 10 to 40% or more. (6), (7), (8), (9), (10).

To secure some information regarding the value of adding skimmilk powder to such mixtures and the amount advisable to add, this work was undertaken.

EXPERIMENTAL

Thirty purebred calves from the Experiment Station herd were used as experimental animals in this study. These calves were not all started on the experiment at the same time, but were started as they were dropped. They were a fairly uniform group and a good representation of normal calves. From birth to eight weeks of age they were allowed to nurse a cow, enough calves nursing one cow so that each calf did not receive more than six to eight pounds of milk daily. At eight weeks of age the calves were weaned to dry grain and hay and divided into three groups of ten calves each. Each group contained seven females and three males.

Group 1, which served as a check group, received a grain ration composed of 100 lbs. wheat bran, 100 lbs. corn meal, 100 lbs. ground oats, and 50 lbs. linseed oil meal.

Group II received the same grain ration as Group I except that 100 lbs. or 22+% of skimmilk powder was added to the ration after the calves were weaned at eight weeks of age.

Group III received the same grain ration as Group I except that 200 lbs. or 36+% of skimmilk powder was added to the ration after the calves were weaned at eight weeks of age. .

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Individual grain feeding was practiced throughout the trial. In order to have results uniform the following grain feeding schedule was rigidly adhered to in the feeding of all three groups.

Birth to 7th week	Nursed cow twice daily Free access to regular grain mixture
7th week to 8th week	Nursed cow once daily Fed 1 lb. grain mixture daily
8th week to 10th week	Fed 2 lbs. grain mixture daily
10th week to 13th week	Fed 3 lbs. grain mixture daily
13th week to 21st week	Fed 4 lbs. grain mixture daily

The grain mixture was supplemented with clover hay (U. S. No. 1 grade), fed *ad libitum.* Fresh water was kept before the calves at all times. They were turned out daily for exercise, when the weather was satisfactory. Salt was made available in boxes in the exercise lots. Daily observations were made of their general condition. Weights were obtained weekly. Photographs were taken of each individual calf at the end of the experimental feeding period for the comparison of growth and development. The results of the feeding trial are based on data collected on each calf from weaning, at eight weeks of age, to twenty one weeks of age, or a period of 91 days.

DISCUSSION

The results obtained in this trial are reported in tables 1 to 4. Table 4 gives a summary of the results obtained. These results give an estimation of the value of adding skimmilk powder to grain rations for dairy calves. An examination of the growth and cost data in this table shows that the calves in Groups I, II, and III made average daily gains of 1.30, 1.38, and 1.49 pounds, respectively. The calves in Group II made an average of 0.08 of a pound and those in Group III 0.19 of a pound more daily gain per calf than was secured per calf in Group I. This increased gain was produced at a cost of $.47 per pound on the calves in Group II and at $.32 per pound on the calves in Group III. The average cost per pound of gain, not including the cost of the roughage fed, for Groups I, II, and III was $.033, $.058 and $.069, respectively.

Throughout the entire experimental period the calves in the three groups continued to appear normal in all outward respects. Several experienced judges were unable to distinguish between the calves of the different groups.

RESULTS

Growth Data For Calves

TABLE 1

Growth data for each calf in Group I—grain ration without skimmilk powder

WEEKS	NUMBER OF CALF									
	347	370	371	427	430	431	436	444	447	452
Weight at birth (lbs.)	80	85	70	70	75	70	80	*	*	90
Weight at 8 wks. (lbs.)	120	155	130	135	130	125	120	170	140	145
9	125	175	135	155	140	140	130	180	145	160
10	130	180	145	160	155	145	140	185	160	165
11	135	200	155	175	165	155	150	195	165	175
12	145	205	165	185	170	165	165	205	175	185
13	140	210	170	190	180	170	165	205	180	185
14	140	225	170	195	185	175	170	220	200	195
15	155	230	180	200	190	180	185	230	205	210
16	165	240	190	205	200	185	195	245	215	220
17	175	245	205	215	200	195	205	250	230	225
18	185	265	210	215	210	210	220	260	235	230
19	195	280	225	215	230	220	225	270	250	250
20	205	290	230	225	240	235	225	280	260	250
21	215	300	240	240	250	245	240	290	270	265
Gain in weight (lbs.)**	95	145	110	105	120	120	120	120	130	120
Av. weekly gain (lbs.)**	7.31	11.15	8.46	8.08	9.23	9.23	9.23	9.23	10.00	9.23
Av. daily gain (lbs.)**	1.04	1.59	1.21	1.15	1.32	1.32	1.32	1.32	1.43	1.32

* Birth weight not obtained.

**For experimental grain feeding period—91 days.

Breed and sex of calves in Group I:

347—Guernsey male
370—Ayrshire ‘‘
371—Jersey ‘‘
427—Ayrshire female
430— ‘‘ ‘‘
431—Guernsey female
436—Ayrshire ‘‘
444—Holstein ‘‘
447— ‘‘ ‘‘
452—Ayrshire ‘‘

TABLE 2

Growth data for each calf in Group II—22 + % of grain ration skimmilk powder

WEEKS	NUMBER OF CALF									
	348	372	376	425	426	428	437	438	445	449
Weight at birth (lbs.)	55	75	80	80	70	70	95	60	*	*
Weight at 8 wks. (lbs.)	90	160	135	145	145	120	140	140	155	155
9	95	165	145	155	155	135	155	145	165	165
10	100	175	160	160	170	145	160	155	175	175
11	105	185	165	160	180	150	175	160	180	190
12	105	195	170	175	190	155	185	170	190	195
13	110	210	175	185	200	165	195	180	200	205
14	115	220	175	190	210	170	205	185	210	215
15	125	230	185	190	220	180	210	195	220	225
16	135	240	200	205	240	195	220	205	235	235
17	150	260	210	220	245	205	235	210	250	250
18	155	275	220	220	250	205	240	210	255	275
19	165	280	230	225	265	215	255	235	260	280
20	175	305	230	230	275	230	265	245	280	290
21	185	310	245	235	285	250	275	250	300	305
Gain in weight (lbs.)**	95	150	110	90	140	130	135	110	145	150
Av. weekly gain (lbs.)**	7.31	11.53	8.46	6.92	10.77	10.00	10.38	8.46	11.15	11.53
Av. daily gain (lbs.)**	1.04	1.65	1.21	.99	1.54	1.43	1.48	1.21	1.59	1.65

* Birth weight not obtained.

**For experimental grain feeding period—91 days.

Breed and sex of calves in Group II:

348—Jersey male
372—Ayrshire male
376—Guernsey "
425—Guernsey female
426—Ayrshire "
428—Ayrshire female
437— " "
438—Jersey "
445—Holstein "
449— " "

TABLE 3

Growth data for each calf in Group III—36 + % of grain ration skimmilk powder

WEEKS	NUMBER OF CALF									
	346	379	383	429	435	446	448	450	454	457
Weight at birth (lbs.)	70	60	100	65	90	*	*	70	70	95
Weight at 8 wks. (lbs.)	120	135	140	120	140	165	150	125	120	145
9	135	145	160	135	150	175	160	130	125	155
10	145	160	165	145	160	190	170	140	130	160
11	155	170	175	155	170	200	180	150	140	160
12	160	180	185	165	180	205	190	160	140	175
13	165	195	195	170	190	225	195	160	155	185
14	180	205	210	185	200	235	205	175	165	195
15	185	215	220	200	215	245	215	190	180	200
16	200	220	225	210	220	255	230	195	190	210
17	210	225	235	230	230	265	245	205	200	220
18	215	235	245	230	235	280	260	215	205	235
19	230	240	255	245	245	290	270	225	220	250
20	240	250	275	255	250	295	290	230	230	255
21	255	270	285	270	260	310	300	245	250	270
Gain in weight (lbs.)**	135	135	145	150	120	145	150	120	130	125
Av. weekly gain (lbs.)**	10.38	10.38	11.15	11.53	9.23	11.15	11.53	9.23	10.00	9.62
Av. daily gain (lbs.)**	1.48	1.48	1.59	1.65	1.32	1.59	1.65	1.32	1.43	1.37

* Birth weight not obtained.

**For experimental grain feeding period—91 days.

Breed and sex of calves in Group III:

346—Guernsey male
379—Jersey male
383—Ayrshire male
429—Ayrshire female
435— “ “

446—Holstein female
448— “ “
450—Guernsey “
454—Jersey “
457—Ayrshire “

TABLE 4

Growth, feed, and cost data for the average calf by groups for the experimental feeding period

GROUP	GAIN IN WEIGHT (LBS.)			LBS. SKIMMILK POWDER FED PER CALF	LBS. GRAIN FED PER CALF	TOTAL LBS. GRAIN AND SKIMMILK POWDER FED PER CALF	COST OF GRAIN AND SKIMMILK POWDER FED PER CALF	COST PER POUND GAIN*
	Total	Weekly	Daily					
I	118.5	9.11	1.30	None	315	315	$3.94	$ .033
II	125.5	9.65	1.38	70.0	245	315	$7.26	$ .058
III	135.5	10.42	1.49	114.5	200.5	315	$9.38	$ .069

* Cost figures do not include roughage fed.
Grain was charged at $25.00 per ton and skimmilk powder at $6.00 per hundred.

CONCLUSION

So far as could be measured by growth-weights and general observations, it was concluded that the increased weight or growth obtained by the addition of a large percentage of dried skimmilk powder to the grain rations of dairy calves, after they had had a good start on milk, incurred more expense than the results would justify.

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A FORMULA FOR EVALUATING FEEDS ON THE BASIS OF DIGESTIBLE NUTRIENTS[1]

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The nutritive value is an important consideration in the selection of a feed, although not the only consideration. The modern feeding standards prescribe the requirements of the cow in terms of digestible crude protein and total digestible nutrients and intelligent feeders appreciate the necessity of supplying the minimum amounts of the two groups of nutrients for good results.

It is common knowledge that feeds vary greatly in composition and in price. It is also factual that high protein feeds as a rule are higher priced than lower protein feeds and that the difference varies from year to year. In the purchase of a feed, therefore, two questions must be answered: First, does it supply the required nutrients, and second, is it a reasonably cheap source of these nutrients?

The problem of economical source of nutrients has long been recognized and attempts have been made to indicate relative cheapness of nutrients in different feeds. One of the most familiar is to charge the entire cost of the feed to either the protein or the total digestible nutrients and express the results in cost per pound. The fallacy of this method is apparent as high protein feeds contain nonprotein nutrients for which it should have credit even though purchased chiefly for its protein content. Likewise feeds purchased chiefly for total digestible nutrients have protein for which they should be credited. The chief objection to this method, however, comes in evaluating medium protein foods where both protein and total digestible nutrient costs become relatively high.

Hayden of Ohio proposed a more accurate, though cumbersome, method for evaluating feeds. It consisted of establishing a value per pound of nutrients in corn by dividing the cost by the total nutrients. Then the value of protein was calculated by:

1. Determining the "excess protein" in linseed oilmeal by calculating all nonprotein nutrients plus sufficient protein to give the same composition as corn. The remaining protein was designated as "excess protein."
2. Crediting linseed oilmeal with its corn equivalent by multiplying the number of pounds by the price established per pound of nutrients in corn.

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3. Subtracting computations of 2 from price of linseed oilmeal.
4. Dividing results of (3) by number of pounds of excess protein.

Hayden designates this as the cost per pound of "excess protein."

It is believed that Hayden's method gives accurate results but is rather too cumbersome for extensive use. The formula hereafter presented has been used by students in feeding at the University of Minnesota and has been found usable and practical. The evaluation is arrived at by using cottonseed meal and corn as the two base feeds, respectively the cheapest source of protein and total digestible nutrients.

One hundred pounds of 43 per cent protein cottonseed meal contains 37.6 pounds digestible protein and 42.6 pounds of nonprotein digestible nutrients. Fifty-seven and twenty-four hundredth pounds No. 2 corn contains 42.6 pounds nonprotein total digestible nutrients and 4.1 pounds digestible protein. Therefore, 57.24 pounds corn can be subtracted from 100 pounds cottonseed meal leaving 33.5 pounds digestible protein. It is reasonable to ascribe any difference in cost between 100 pounds cottonseed meal and 57.24 pounds corn to the 33.5 pounds digestible protein and by simple division arrive at the value of 1 pound of digestible protein. To find the value of the nonprotein total digestible nutrients, credit either feed with its protein value and the difference from the cost of the feed is charged to the nonprotein digestible nutrients.

The formula for determining the cost for a pound of protein is:

$$\frac{\text{Cost of 100 pounds cottonseed meal} - .5724\ (\text{cost of 100 pounds corn})}{33.5}$$

Example: Substituting with cottonseed meal at $50.00 per ton and corn at $25.00 per ton

$$\frac{\$2.50 - (.5724 \times \$1.25)}{33.5} = 5.328 \text{ cents}$$

The value of a pound of nonprotein digestible nutrients may be arrived at by the following formula

$$\frac{\text{Cost of 57.24 pounds corn} - (\text{value of protein} \times 4.1)}{42.6}$$

Again substituting with corn at $1.25 per hundredweight and protein at 4.328 cents per pound

$$\frac{1.25 \times 57.24 - (4.1 \times 5.328)}{42.6}$$

or 1.167 cents per pound.

Should some other feeds be a cheaper source of either protein or nonprotein nutrient such feeds may be used to establish the value of either protein or nonprotein nutrients. This however is seldom necessary as by using corn and cottonseed meal as base feeds other cheaper feeds would be indicated, as selling for less than their value on the basis of corn and cottonseed meal.

By applying these values to the digestible protein and nonprotein digestible nutrients of other feeds their values are arrived at. It is apparent that for a given feed both cottonseed meal and corn exert a definite influence on its value and therefore constants can be determined for each

TABLE 1

Giving constants by which corn and cottonseed meal prices must be multiplied to give values of more common feeds

	CONSTANT		VALUE WHEN	
	For cottonseed meal	For corn	Corn is $20.00 and cottonseed meal $40.00	Corn is $20.00 and cottonseed meal $30.00
Oats	.116	.749	$19.62	$18.46
Wheat	.062	.909	20.68	20.04
Barley	.067	.907	20.82	20.15
Rye	.064	.940	21.38	20.72
Soybeans	.709	.342	35.23	28.11
Flaxseed	.380	.885	32.90	29.10
Corn and cottonseed meal	.024	.980	18.64	18.88
Bran	.222	.512	19.18	16.90
Middlings	.240	.613	21.86	19.46
Linseed oilmeal	.765	.207	34.74	27.09
Gluten meal	1.043	-.040	40.92	30.49
Gluten feed	.466	.532	29.27	24.62
Soybean meal	1.054	.010	42.21	31.62
Beet pulp	- .054	.930	16.44	16.98
Molasses	- .137	.863	11.78	13.15
Mangels	.014	.065	1.86	1.72
Potatoes	- .013	.225	3.98	4.11
Alfalfa	.201	.427	16.58	14.57
Red clover	.100	.509	14.18	13.18
Sweet clover	.212	.413	16.77	14.62
Mixed hay	.026	.578	12.60	12.34
Timothy	- .049	.647	10.98	11.47
Prairie	- .040	.630	11.00	11.40
Corn fodder	- .016	.603	11.42	11.58
Corn stover	- .039	.484	8.12	8.51
Corn silage	- .008	.214	3.96	4.04
Oat straw	- .098	.655	9.20	10.18

How to use the table:

Look for constants under head "For cottonseed meal" following the feed, the value of which is desired. Multiply the price of cottonseed meal per ton by this factor, then multiply the price of corn per ton by factor found under head, "For corn." Add the two products which is the value per ton of the feed under consideration.

Note—If the constant is preceded by a - (minus) sign, subtract in place of adding.

feed which when applied to the prices of cottonseed meal and corn should give the correct value of the feed.

By calculating the values of a feed with corn constant and cottonseed meal at two different values a constant can be determined by which cottonseed meal may be multiplied to indicate the influence upon the price of the feed. Likewise constants may be determined for corn.

Example: Using previous formulae oats have a value of $19.61 per ton when cottonseed meal is $40.00 and corn $20.00 per ton, and $18.45 per ton when corn is $20.00 and cottonseed meal $30.00 per ton. Therefore a $10.00 increase in cottonseed meal increases the value of oats $1.16.

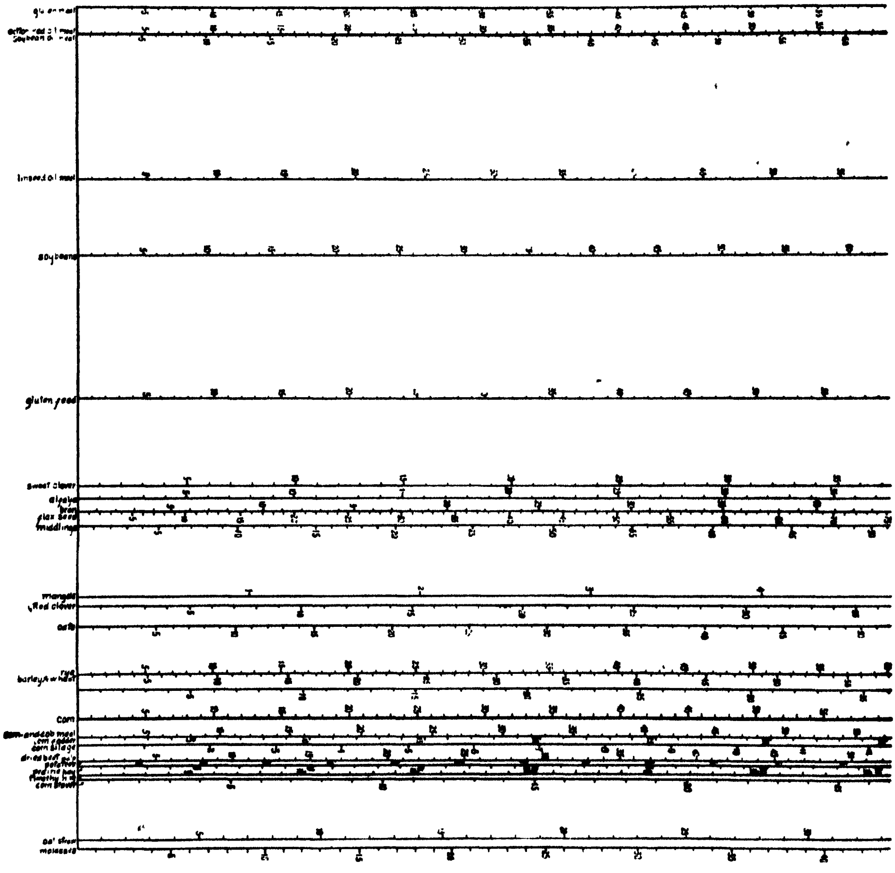

FIG. 1

Chart for evaluation of feeds upon basis of value of corn and cottonseed meal. To determine the value per ton of any feed on the chart place straight edge on value of corn, the heavy line toward the right, and on cottonseed meal, the heavy line toward the left. The point of intersection on the scale for any feed gives its value in dollars per ton.

Keeping the price of cottonseed meal constant and increasing the price of corn we find that a $10.00 increase in price of corn increases the value of oats $7.49. For any price therefore the value of oats = .116 × price of cottonseed meal + .749 × price of corn.

The following table gives the constants by which cottonseed meal prices and corn prices must be multiplied to give the values of the more common feeds. Values with corn at $20.00 and cottonseed meal at $30.00, and corn at $20.00 and cottonseed meal at $40.00 are given.

A GRAPH FOR DETERMINING VALUE OF COMMON FEEDS

It also becomes apparent a graph can be constructed by which the value of any feed can be determined on the basis of values of corn and cottonseed meal. This is done in figure 1. This graph is constructed by placing the proper divided scale for any feed in the proper place between corn and cottonseed meal. The scale is ascertained by determining the value of that feed when corn and cottonseed meal are $60.00 per ton each, and dividing into the proper sectors. The location of the scale is determined by the relative influence of corn and cottonseed meal upon the value of the feed such scale represents.

THE RELATION OF THE SOLUBLE PORTION OF ALFALFA TO THE RAPID ABSORPTION OF FEED FLAVOR IN MILK

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Feed as a source of flavor in milk has been known for more than a century (1) and the absorption of feed flavors in milk soon after the feed has been consumed has been reported by investigators in recent times (2) to (6). Although it has been generally accepted that feed is an important source of flavor in milk when given to cows previous to milking, it has not been previously described how the feed flavor could appear in the milk 20–30 minutes after the ingestion of the feed, since the feed ordinarily remains in the rumen several hours before it passes on to the abomasum or fourth stomach and the intestinal tract where absorption takes place. Schalk and Amadon (7) in their study of the physiology of rumination pointed out that as soon as roughage was swallowed by the animal and passed into the rumen it was flooded with waves of liquid forced into the rumen from the second stomach. This action takes place with each peristaltic contraction of the rumen. This offered to the writers a possible explanation for the presence of the feed flavor in milk soon after flavor-producing feeds were consumed by the animal. The present experiment was undertaken to study the rate at which the juice of the alfalfa plant made its appearance as a feed flavor in the milk.

EXPERIMENTAL

Green alfalfa, cut just before the bloom stage, was immediately placed in the cold storage room at 15° F. and thoroughly frozen. The purpose of the freezing was to rupture the plant cell walls, thus aiding in the extraction of juice from the plant tissues (8). After thawing the alfalfa at room temperature, it was cut into fine pieces by the use of a food grinder and the liquid pressed out of the pulp and filtered to remove all solid particles. This juice was then administered to the cows as a drench. The liquid from 25 pounds of alfalfa amounted to between 5 and 6 quarts and this quantity was used in each case as the standard drench. It required 3½ to 5 minutes for 2 men to administer the juice. Cows producing milk with good flavor were selected from the University Farm Herd, and all feed was withheld from the cows for five hours previous to milking in order to eliminate extraneous sources of feed flavor.

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The cows were partially milked before drenching in order to secure a sample with normal flavor. Other portions of the milk in the udder were drawn every five minutes after drenching for 30 minutes. In some instances, however, samples were drawn at the end of 45 minutes, one hour, and two hours following drenching. The milk samples collected were scored for flavor within two hours after the last sample had been drawn. The samples were numbered in such a way that their identity was unknown to the judges.

TABLE 1

Flavor Comments on Milk of Cow Number 153 Before and After Administration of Alfalfa Juice

INTERVAL AFTER DRENCHING BEFORE MILK SAMPLES WERE DRAWN (IN MINUTES)	SCORE	COMMENTS
Control-sample taken before drenching*	13.5	No feed flavor
5	13.5	No feed flavor
10	13.4	No feed flavor—slightly astringent
15	13.4	No feed flavor—slightly astringent
20	13.3	Slight feed flavor
25	13.2	Feed flavor and odor
30	13.2	Feed flavor and odor
Control-sample taken before drenching**		No feed flavor
5		No feed flavor
10		No feed flavor
15		Slight odor. No feed flavor
20		Very slight feed flavor
25		Slight feed flavor and odor
30		Slight feed flavor and odor
60		Distinct feed flavor and odor

* TRIAL I—Cow No. 153—5 quarts of liquid from 25 pounds of alfalfa used as a drench. Time required to drench 5 minutes. Cow was maintained on oat hay and concentrate rations. No feed given 5 hours before drenching.

** TRIAL II—Cow No. 153—6 quarts of liquid from 25 pounds of alfalfa used as a drench. Time required to administer drench 5 minutes. Cow maintained on alfalfa and concentrate rations. No feed given 5 hours before drenching.

DISCUSSION OF RESULTS

The data show that twenty minutes after drenching the cows with juice expressed from the green alfalfa plant, feed flavor was present in the milk. It had been observed that feed flavor appears in the milk 20 to 30 minutes after a cow has eaten 15 to 20 pounds of green alfalfa (6). This experiment indicates that the flavor-producing materials are present in the liquid that is expressed from the feed during partial mastication in the mouth and released during the maceration process in the rumen. In the case of

TABLE 2

Flavor Comments on Milk of Cows Numbers 441 and 428 Before and After Administration of Alfalfa Juice

INTERVAL AFTER DRENCHING BEFORE MILK SAMPLES WERE DRAWN (IN MINUTES)	SCORE	COMMENTS
Control-sample taken before drenching*		No feed flavor
5		No feed flavor
10		No feed flavor
15		Very slight feed flavor
20		Slight feed flavor and odor
25		Definite feed flavor and odor
30		Slight feed flavor and odor
60		Strong feed flavor and odor
120		Distinct feed flavor and odor
Control-sample taken before drenching**	13.5	No feed flavor
5	13.5	No feed flavor
10	13.5	No feed flavor
15	13.5	No feed flavor
20	13.2	Slight feed flavor and odor
30	13.1	Feed flavor and odor
45	13.0	Definite feed flavor and odor
60	13.2	Definite feed flavor and odor
120	13.4	Very slight feed flavor and odor

* Cow No. 441—5 quarts of liquid expressed from 25 pounds of alfalfa used as a drench. Time required to drench 5 minutes. No feed given 5 hours before drenching. Cow maintained on oat hay and concentrate rations. The one-hour sample had a more prominent feed flavor than the two-hour samples.

** Cow No. 428—5 quarts of liquid expressed from 25 pounds of alfalfa was used as a drench. Cow was maintained on alfalfa and concentrate rations. No feed received 5 hours before drenching.

dry feed, such as alfalfa or clover hay, in which there is considerable flavor producing material, the waves of liquid passing over the feed in the rumen after it is swallowed apparently extract a portion of the flavor producing materials from the feed and carry them on through the digestive tract where they are rapidly absorbed. Normally the feed eaten by ruminants remains in the rumen for a considerable time before it is returned to the mouth for final mastication and passage on through the alimentary tract for absorption. Most of the liquid passes on immediately.

The rapid absorption of the juices rather than the solid portions of the feed, would seem to be concerned with the early absorption of feed flavor in milk. It was found with alfalfa juice that the most prominent feed flavor was present in samples drawn 45 to 60 minutes after drenching.

In previous experiments where green or dry alfalfa was given to cows the most prominent feed flavor was observed in the milk two hours after the feed was placed before the cows. It is apparent that the juice of the alfalfa plant carries a considerable portion of the flavor-producing materials and that the administration of this juice as a drench leads to more rapid absorption by the animal.

CONCLUSIONS

(1) Feed flavor appears in the milk twenty minutes after the ingestion of flavor-producing materials in liquid form.

(2) The most pronounced feed flavor was present in the milk drawn 45 to 60 minutes after drenching.

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A STUDY OF METHODS USED IN CONDUCTING A SILAGE FEEDING EXPERIMENT

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This paper deals with the methods employed in the conduct and analysis of the data of the silage feeding experiment reported in Bulletin 169 of the Storrs Station. (1)

Data are presented which justify the use of long experimental periods, a practice that is becoming well established in later years.

An analysis is presented of a method of weighing which gives great accuracy and eliminates forty per cent of the labor of weighing. Data are also presented which indicate equal accuracy in morning and afternoon weighing.

A method is presented of measuring the efficiency of milk production of experimental animals.

LENGTH OF PERIODS

In the silage experimental work of this station it has been the practice to provide a 20 day preliminary period to precede the 100 day experimental period. The experimental period was intended to be 100 days in length in each trial of this experiment but the second trial was discontinued at 70 days because of the advanced stage of lactation of most of the cows. A study of average group production for the individual or combined trials does not show any consistent change in efficiency (the method of determining efficiency is shown on page 308) which would have affected the results had the preliminary period been but 10 days. The efficiency of both groups would have been higher had the experimental period been 30 instead of 100 days but it would not have affected the comparison of the two groups, the light silage group would still have produced more efficiently. A study of the data for individuals, however, shows that some animals do not adjust themselves to a change quickly enough that a preliminary period of less than 20 days is justified. This is especially true if the ration is not properly adjusted at the start to the capacity of the cow. An example of this is seen in the daily feed record of Korndyke for the first 30 days of the first trial which is recorded in table 1.

For the first two days Korndyke ate nearly 10 pounds of hay at each feeding but refused on the fifth feeding and had her hay reduced to 9

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TABLE 1

Daily food consumption of Korndyke

DAY OF EXPERIMENT	GRAIN	SILAGE	HAY	
			Fed	Refused
	pounds	*pounds*	*pounds*	*pounds*
1	10	18	20.0	0.7
2	10	18	20.0	0.3
3	10	18	19.0	3.8
4	10	18	18.0	0.4
5	10	18	18.0	0.0
6	10	18	18.0	0.0
7	10	18	18.0	0.4
8	10	18	18.0	0.3
9	10	18	18.0	0.3
10	10	18	18.0	0.1
11	9.9	18	18.0	0.3
12	9.8	18	18.0	0.7
13	9.6	18	18.0	0.1
14	9.4	18	18.0	0.2
15	9.2	18	18.0	0.1
16	9.0	18	18.0	0.6
17	8.8	18	18.0	2.0
18	8.6	18	15.0	4.4
19	8.4	18	12.0	0.0
20	8.2	18	12.0	0.0
21	8.0	18	12.0	0.0
22	8.0	18	12.5	0.0
23	8.0	18	13.0	0.0
24	8.0	18	13.5	0.0
25	8.0	18	14.0	0.1
26	8.0	18	14.0	0.1
27	8.0	18	14.1	0.0
28	8.0	18	14.2	0.0
29	8.0	18	14.2	0.0
30	8.0	18	14.2	0.0

pounds which she consumed for 14 days refusing on an average 0.2 pounds until on the 18th day she refused 4.4 pounds of hay. The hay was then reduced to 6 pounds per feeding for three days, after which it was increased to 6.5 pounds for two days and then finally to 7 pounds at each feeding (14 pounds daily) which she continued to consume for the remainder of the experiment. Had she received 8 pounds or 8.5 pounds at first she might have continued to consume that for 30 days or more without refusing whereas her actual capacity was approximately 7 pounds of hay with the silage and grain which she received. It is of interest that her weight fell off shorply for twelve days in spite of the fact that she was being overfed and then fluctuated to the end of the 20 day preliminary period when it became comparatively stable. It will be noted that the second reduction in hay consumption occurred despite a parallel reduction in grain. There would have been a marked effect upon the feed consumption if these data had been for the second or reversed period of an experiment of the reversal type with the first seven days acting as a 7 day interval.

WEIGHING

In the previous experiments of this station it has been the practice to weigh all animals daily to the nearest pound. In these experiments the main purpose of the weights has been to guide the feeding practice.

Three successive days weights were averaged (the fourth and fifth omitted) for each five day period of a previous experiment and the resulting averages plotted against the average of five daily weights. Changes in the feeding program of the experiment utilized for this comparison had been dependent upon averages of daily weights for the previous 10 days. It was thought the method would be open to criticism if not based upon weights of animals whose changes in feeding program had been dependent upon an average of three successive days weights for each five day period. Lush (2) has averaged three successive days weights in analyzing growth experiments. If comparative gains in the weights of two groups are being studied there is much greater need of accuracy than in this experiment where weight change was but one of the criteria which determined changes in the ration.

In the first feeding trial of this experiment the cattle were weighed daily at 8 A. M. to the nearest pound. An analysis of the weights obtained is shown in table 2.

TABLE 2

A comparison of daily weighing with weighing three successive days of each five day period

	DAILY WEIGHING	WEIGHING THREE SUCCESSIVE DAYS OF EACH FIVE-DAY PERIOD
Mean weight (lbs.)	1113.	1113.
Standard deviation (lbs.)	11.8	11.7
Probable error (lbs.)	± 7.96	± 7.89
Percentage coefficient of variability	1.06	1.05

The weights of the entire group of cows, eight in number, were analyzed for the experimental period of 100 days, or a total of 800 weighings. It will be noted that the mean weight was the same by both methods and that the probable errors and percentages coefficients of variability differed but slightly. This indicates a greater degree of accuracy than that obtained by Lush and Black (3) who state that "The error of estimate from a 3 days weight was 58% as large as from a 1 day weight. Ten weighings would be required to reduce the error to one third what it was from a single weight." Accordingly, the animals were weighed but three days of each five day period of the second and third trials.

In the third trial afternoon weighing was more convenient. The group of ten animals were weighed both at 8 A. M. and at 1 P. M. three successive days in each 5 day period for thirty days. A comparison of morning and afternoon weighing is presented in table 3. The data are based upon eighteen weighings for each animal, or a total of 180 weights for both morning and afternoon. No consistent differences can be observed in the probable error or percentage coefficients of variability for individual animals between morning and afternoon weighings. When the Heavy Silage and Light Silage Groups are considered as entities the magnitude of the probable errors and percentage coefficients of variability is much smaller than for individuals due to the greater number of weighings considered. When both groups are considered collectively the probable error is reduced to ± 2.7 pounds and the percentage coefficient of variability to 0.41 for both morning and afternoon weighing. By comparing the probable errors and percentage coefficients of variability for individual animals with those of the two groups and for the average of both groups it will be seen that

TABLE 3

A comparison of morning and afternoon weighing

	8 A. M.			1 P. M.		
	Mean weight	Probable error	Percentage coefficient of variability	Mean weight	Probable error	Percentage coefficient of variability
Heavy silage group	*pounds*	*pounds*		*pounds*	*pounds*	
Queen	1371	± 7.2	.92	1346	± 6.1	.68
Secret	970	± 4.1	.63	955	± 4.0	.62
Laureate	1123	± 6.2	.82	1101	± 8.0	1.08
Jasmine	826	± 4.9	.87	810	± 3.8	.70
Favorite	774	± 6.0	1.15	768	± 6.1	1.18
Group average	1013	± 3.4	.49	996	± 3.4	.50
Light silage group						
Korndyke	1187	± 6.3	.78	1160	± 7.9	1.00
Graceful	1008	± 4.5	.66	989	± 4.7	.70
Amiable	1090	± 4.7	.64	1083	± 5.3	.73
Tontine	874	± 4.1	.70	862	± 3.4	.58
Brilliant	747	± 3.7	.74	734	± 4.7	.94
Group average	981	± 3.3	.40	966	± 3.2	.49
Average of both groups	997	± 2.7	.41	981	± 2.7	.41

the deviations from the mean are compensating rather than cumulative. This indicates careful control of experimental conditions.

The absence of any considerable differences between the Heavy Silage and Light Silage Groups in these respects indicates that the type of ration did not alter the accuracy as between morning and afternoon weighing.

Afternoon weighing is therefore as accurate as morning weighing but the mean morning weight was 16 pounds greater than the afternoon weight. This difference in weight is significant since it is six times as great as the probable error. This difference in weight can be accounted for when it is considered that the cows were fed silage and grain at 6 A. M. followed by hay feeding. Water was before them in a water cup and they drank following hay feeding prior to the 8 A. M. weighing. From 8 A. M. to 1 P. M. they drank proportionately less and lost weight by excretion and respiration. The difference in the mean morning and afternoon weights emphasizes the necessity of weighing at the same hour each day.

EFFICIENCY OF PRODUCTION

For a comparative experiment an equable basis of comparison is imperative. In this experiment it is desirable to relate milk production to dry matter consumption for each group. Solids not fat, butterfat, or even

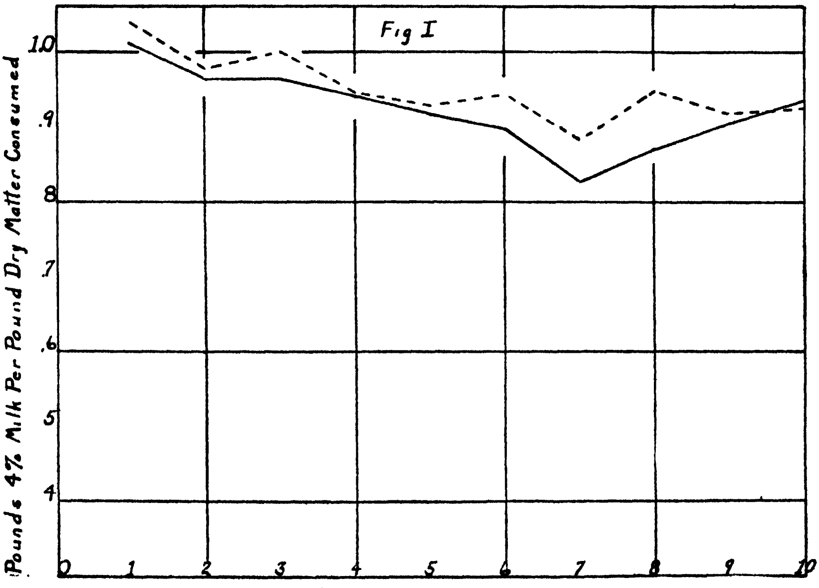

FIG. I. POUNDS OF 4 PER CENT MILK PER POUND OF DRY MATTER CONSUMED BY THE TWO GROUPS BY 10-DAY PERIODS.

Heavy Silage Group ————————.

Light Silage Group - - - - - - - -.

total solids is not satisfactory as a basis of comparison since the total energy content is not considered. Gaines and Davidson (4) have derived a formula by which actual production can be converted on an energy basis to an equivalent amount of 4 per cent milk. This formula ($.4 \times \text{milk} + 15 \times \text{fat} = \text{equivalent 4 per cent milk}$) has been used to convert the production to 4 per cent milk, which is a satisfactory basis of comparison for this purpose. The amounts of 4 per cent milk produced and of dry matter consumed have been calculated for 10 day periods for each animal, and ratios of 4 per cent milk per pound of dry matter have been computed.

These data are plotted in Figure I. Only a small difference in efficiency is noted between the two groups; however a decided decrease in efficiency with advance in lactation is evident in both groups. In order to relate this decrease in efficiency to the time of calving, the data were tabulated by 10 day periods and distributed according to the date of calving and the ratios for efficiency again computed. (Data relative to calving date are presented in Bulletin 169 of the Storrs Station.) These ratios of efficiency are plotted in Figure II. Decrease in efficiency with advance in lactation becomes more pronounced when the data are plotted in this manner. The differences between the two groups are too meager and irregular to be significant with respect to type of ration. A study of the data from which Figure II was derived shows that not more than 8 animals are represented at any one 10 day period in the table, far too few numbers to establish a curve from which interpretations involving small differences may be made.

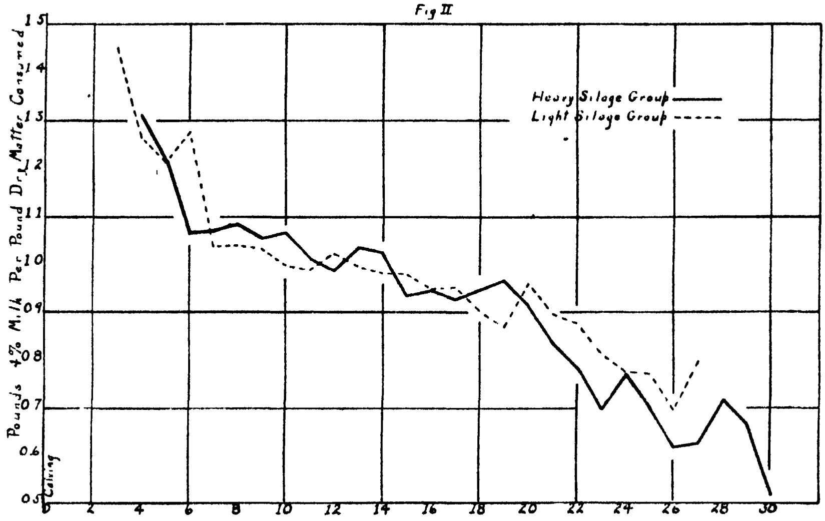

FIG. II. POUNDS OF 4 PER CENT MILK PRODUCED PER POUND OF DRY MATTER CONSUMED, PLOTTED ACCORDING TO THE LENGTH OF TIME AFTER CALVING.

Eckles (5) gives data showing what percentage of the production of the maximum month of lactation is produced in succeeding months. When these data are plotted against the weighted averages of the 10-day periods of each month following calving for both groups collectively the relationship shown graphically in Figure III is obtained.

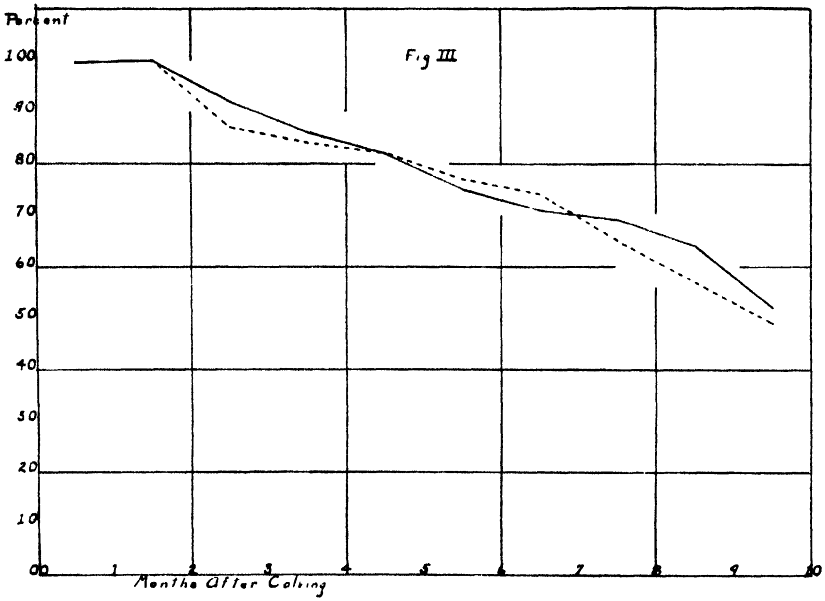

FIG. III.

Eckle's Data ———————— Decline in Milk Production with Advance in Lactation.
Storr's Data - - - - - - - - - Decline in Efficiency of Production with Advance in Lactation.

Error from this source was avoided as far as possible by carefully distributing the animals within the two groups with respect to stage of lactation.

It seems very probable that the decline in efficiency with advance in lactation is at least partially due to the increasing proportion of the dry matter consumption that is required for maintenance. The tendency manifested by all milking cows to lose weight in the early part of the lactation period and to regain it later has undoubtedly added to the decline in efficiency with advance in lactation. That decline in weight is comparatively small in this experiment is shown by the fact that the daily decline for the Heavy Silage Group was one twentieth of a pound per cow day and for the Light Silage Group one thirteenth pound.

Ratios of efficiency for individuals and groups for each feeding trial are shown in table 4. The data is concordant in showing a slightly greater efficiency for light silage feeding in each of the three trials in spite of

TABLE 4

Pounds of 4 per cent. milk produced per pound of dry matter in the ration

	HEAVY SILAGE GROUP						LIGHT SILAGE GROUP					
Trial I	Delight	Senora	Amiable	Sagacious		Group	Korndyke	Queen	Buoyant	Laureate		Group
	.926	.862	.888	1.105		.943	.808	.807	1.346	.991		.987
Trial II	Martha	Vanity	Genuine	Juniper		Group	Graceful	Secret	Poppy	Magnetic		Group
	.761	1.149	.595	.757		.847	.899	.946	.900	.820		.885
Trial III	Queen	Laureate	Secret	Jasmine	Favorite	Group	Korndyke	Amiable	Graceful	Tontine	Brilliant	Group
	.806	.877	.817	1.188	1.168	.955	.958	.837	.837	.941	1.153	.969
Weighted average of three trials						.925						.953

variations in quality of silage and hay and varying stages of lactation. It is evident from the wide variation in individual efficiency that drawing conclusions from small numbers and short experimental periods is not justified.

Reference to the body weights of the animals for the three feeding trials described (1) shows that collectively the Light Silage Group weighed 68 pounds more than the Heavy Silage Group. Since for the three trials the animals were on experiment an average of 88 days, the Therms of energy required for maintenance might be calculated and a correction be made. The maintenance requirement would be 36.32 Therms.

Further, since the weight changes of the two groups for the three trials collectively were 100 pounds in favor of the Heavy Silage Group a correction of 325 Therms should be made in its favor.

The algebraic sum of these two corrections would be 288.28 Therms in favor of the Heavy Silage Group. Since one pound of 4 per cent milk contains 0.336 Therms of energy this would equal 858.0 pounds of 4 per cent milk. If this amount be added to the corrected 4 per cent milk produced by the Heavy Silage Group for the three trials and the ratio of pounds of 4 per cent milk per pound of dry matter be calculated we find a ratio of 0.956 (that for the Light Silage Group was 0.953). Obviously, this is within experimental error. Therefore, it must be concluded that there is no difference in efficiency in use of dry matter between the two groups.

SUMMARY

Experimental data are submitted to show the undesirability of short experimental periods and the dangers of the reversal type of experiment.

An analysis of the daily weights of a group of eight animals for a 100 day period shows that weighing three successive days of each 5 day period is practically as satisfactory as weighing daily.

Statistical analysis of morning and afternoon weighings shows that these animals averaged 16 pounds heavier at 8 A. M. than at 1 P. M. but shows no significant difference in probable error or percentage coefficients of variability at these hours.

The ratios of pounds of 4 per cent milk per pound of dry matter in the ration are given for individuals and for groups with advance in lactation. A marked decline in efficiency is shown with advance in lactation.

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METHOD FOR OBTAINING REPRESENTATIVE SAMPLES OF BLOOD FOR INORGANIC PHOSPHORUS IN DAIRY HEIFERS[1]

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In recent years much study has been devoted to the inorganic phosphorus of the blood, because of its relation to various problems of nutrition, metabolism, and diseases.

Howard and Reay (4) report that the concentration of blood phosphorus is quite variable in human blood examined at hourly intervals. When the subject was changed from complete rest to ordinary movements about the laboratory, large fluctuations were observed. Robinson and Huffman (8) found a variation of inorganic phosphorus in blood of beef animals as high as 1.87 mg. per 100 ml. of plasma in 24 hours, and as much as 2.89 mg. during the 8 days during which daily determinations were made. Palmer, Cunningham, and Eckles (6) noted that blood phosphate in individual cattle may vary markedly from day to day even when the blood is drawn under apparently identical conditions. They also noted that the inorganic phosphate in individual cattle may vary from hour to hour. Certain physiological factors such as the ration (7), the ingestion of soluble carbohydrate (2), parturition (5), (8), (6), and exercise (6) have been shown to affect blood phosphate.

In early investigations of the inorganic phosphorus of the blood, single samples were used to obtain representative values for individual animals. Because the inorganic phosphorus of the blood is subject to day-to-day fluctuations, Palmer, Cunningham, and Eckles (6) and Henderson and Weakley (3) used the mean for three consecutive days as a representative phosphorus value.

In earlier work at this Station (3), when heifers were fed on both high and low-phosphorus rations, even though samples were taken on three consecutive days, marked variations were obtained from one determination to the next. These variations in blood composition led the authors to try to improve their method of sampling.

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EXPERIMENTAL

Samples of blood of about 15 ml. from the jugular veins of dairy heifers were collected in test-tubes containing 2.5 mg. of potassium oxalate per ml. as an anticoagulant. During warm weather the samples were immediately placed in crushed ice, carried to the refrigerator, and stored at 2° C. until analyzed for phosphorus. Analyses were made in most cases on the day the samples were taken; otherwise on the day following. All samples were taken at about 9 o'clock in the morning, approximately two hours after the heifers had been fed and watered. Inorganic phosphorus was determined on the oxalated blood by the method of Fiske and Subbarrow (1).

Daily Variations

The values plotted in figure 1 were obtained on 12 consecutive days on each of 8 animals. They show the extent to which the inorganic phosphorus may vary in 24 hours. It will be noted that a variation of 1.72 mg. per 100 ml. of blood occurred during 24 hours, and a variation as high as 2.34 mg. per 100 ml. of blood occurred during the 12 days in which daily determinations were made. The average daily variation for the 8 animals during the 12-day period was 0.46 mg. per 100 ml. of blood. It is possible that still greater variations may occur. Marked fluctuation from one day to the next is not unusual. However, in some instances a gradual rise was observed for a period of several days, followed by a slow decline. There seems, however, to be no consistency in the variations from one period to the next which might indicate cyclic or periodic fluctuations.

In order to obtain representative samples of blood the ideal method would be to sample several times daily for a period of several days. This procedure would require a considerable amount of time and might affect the animals adversely. When daily samples were taken for a prolonged period, knots and sensitive areas were developed above the jugular vein where the needle was inserted. This practice seemed to make the animals nervous and more easily excited. An attempt therefore was made to obtain a representative value of the inorganic phosphorus of the blood from the least number of samples and with the least possible disturbance of the animal.

When a study was made of the values obtained from daily sampling (Fig. 1) it was found that the mean of alternate-day sampling would check very closely with the mean of all 12 samples taken on 12 consecutive days, as shown in table 1. In this table are listed also values obtained for each three-consecutive-day period, as for the first three days, the second three days, etc. This was the method used at this Station in earlier work (3) and by other investigators (6). The deviations from the means of the 12-consecutive-day period shows that alternate-day sampling is much to be preferred over the values obtained for the three-consecutive-day periods.

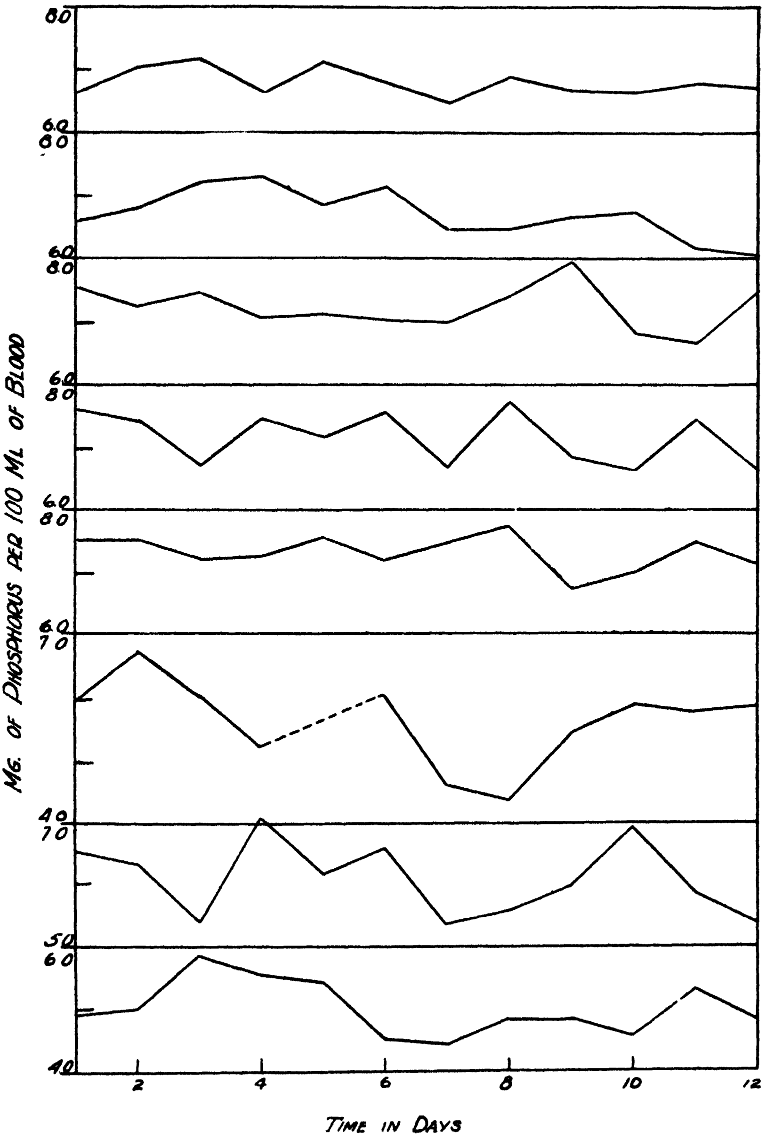

FIG. 1. DAILY VARIATIONS IN INORGANIC PHOSPHORUS OF BLOOD

Length of Storage Period

A study followed to determine the length of time the blood could be kept in storage and still give reliable results when analyzed for inorganic phosphorus. Suitable storage periods would provide for the possibility of obtaining a composite sample made up of a large number of individual samples on which a value might be obtained, which would be representative

TABLE 1

Milligrams of inorganic phosphorus per 100 ml. of blood

NUMBER OF ANIMALS	E35	E36	E37	E38	273	290	301	306	RANGE OF DERIVATION FROM 12-DAY MEAN (MGS.)
Means of:	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Twelve consecutive days	6.79	5.01	6.70	7.24	5.62	6.07	7.13	7.27	
Six alternate even days	6.80	5.13	6.66	7.30	5.56	5.86	7.00	7.29	−0.21 to +0.12
Six alternate odd days	6.79	4.89	6.74	7.17	5.67	6.29	7.22	7.27	−0.12 to +0.22
1st three days	6.94	5.25	6.87	7.44	6.11	6.06	7.22	7.37	−0.01 to +0.49
2nd " "	6.84	5.19	7.09	7.07	5.62	6.60	7.37	7.31	−0.17 to +0.53
3rd " "	6.96	4.70	6.51	7.44	4.80	5.61	7.06	7.28	−0.82 to +0.20
4th " "	6.71	4.90	6.32	6.99	5.83	6.02	6.88	7.15	−0.28 to +0.21

of the average content of that constituent in the blood of the individual animal.

Samples of about 200 ml. of blood were collected and divided into 15 aliquots of about 12 ml. each. The aliquots were stored in the refrigerator at 2° C. until they were analyzed for inorganic phosphorus.

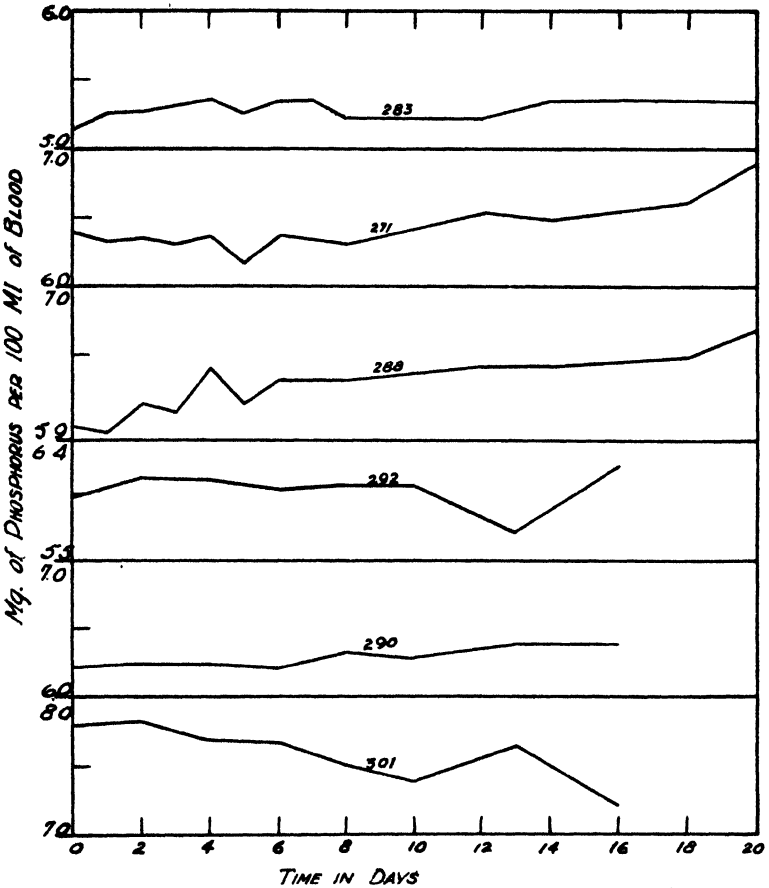

FIG. 2. CHANGES IN THE INORGANIC PHOSPHORUS OF BLOOD IN STORAGE

It may be noted from figure 2 that when these samples were analyzed there were no significant changes in the inorganic phosphorus until after a storage period of about two weeks. After a storage period of more than two weeks slightly higher values were obtained in most cases, due probably to decomposition of compounds containing phosphorus in organic combination.

From the data in figure 2 it was concluded that reliable values for inorganic phosphorus of blood in storage could be obtained after a storage period of two weeks.

Method of Obtaining Representative Samples

In order to obtain representative values for the inorganic phosphorus content of the blood for the individual animals, samples of blood of about 15 ml. were taken on alternate days for a period of 12 days. The samples were kept in storage at 2° C. until all six samples had been taken. They were then removed from the refrigerator and allowed to come to room temperature (about 20° C.). After the samples were thoroughly mixed by rotating the tube in such a way as to keep the blood from the mouth of the tubes, a composite sample of blood was prepared by taking 10 ml. from each of the six samples. This composite sample was thoroughly mixed by pouring from one beaker to another. Then it was analyzed for inorganic phosphorus by the method of Fiske and Subbarrow (1).

This procedure has been in use for several months in a study at this Station of the effect of feeding rations containing various amounts of phosphorus to dairy heifers. Samples have been taken on alternate days over a 12-day period during the first half of each month, and composites of these have been analyzed. No ill effects from the number of samples taken have been observed. In table 2 are listed some determinations made on the blood of growing dairy heifers which were fed a normal ration. A certain amount of fluctuation from one month to another is noted, but with this method of sampling the variations are not nearly so great as have

TABLE 2

Inorganic phosphorus in blood of normally fed animals (mg. of P. per 100 ml. of blood)

DATE OF SAMPLING	NUMBER OF ANIMAL			
	E35	E38	E39	E40
4 (14–24) '30	6.48	7.20	7.02	7.98
5 (12–22) '30	6.60	6.93	7.44	7.68
6 (2–12) '30	6.23	7.15	7.26	7.53
7 (1–11) '30	6.38	7.20	7.64	7.42
8 (2–12) '30	6.03	6.72	7.48	7.10
9 (2–13) '30	5.96	6.93	6.60	7.38
10 (7–17) '30	5.30	6.12	6.48	7.08
11 (4–14) '30	5.28	5.73	6.16	6.57
12 (2–12) '30	5.28	4.92	5.52	6.15
1 (6–16) '31	4.50	5.42	6.31	5.35
2 (3–13) '31	5.63	5.21	6.41	7.12
3 (3–13) '31	5.82	5.21	5.78	7.03
4 (3–13) '31	5.46	5.24	6.08	6.71
5 (4–14) '31	5.36	4.67	5.94	6.32
6 (3–13) '31	5.18	4.56	5.45	6.36
7 (2–12) '31	5.81	4.89	5.60	6.24

been obtained from one day to another. The average monthly range in variation over a period of 16 months for the four animals (Table 2) is 0.35 mg. per 100 ml. of blood.

SUMMARY

The inorganic phosphorus content of the blood of dairy heifers was found to vary considerably from day to day. The average daily variation of eight dairy heifers was found to be 0.46 mg. per 100 ml. of blood for a 12-day period. However, a variation of 1.72 mg. per 100 ml. of blood for 24 hours occurred, and a variation as high as 2.34 mg. per 100 ml. of blood for the 12 days during which daily determinations were made was found.

Alternate-day sampling over a period of 12 days proved to be the most satisfactory method studied for obtaining a representative sample. The average monthly range in variation as determined by this procedure for four animals over a period of 16 months was found to be 0.35 mg. per 100 ml. of blood.

Storage of blood at 2° C. for 2 weeks was shown to have no significant effect on the amount of inorganic phosphorus found in the blood.

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THE COLI-AEROGENES DETERMINATION IN PASTEURIZATION CONTROL

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Central control of pasteurization of milk and cream involves three essential factors: supervision of design and installation of plant equipment, inspection of plant operation, and laboratory examination of the product at various stages of the processing. During the past few years particular attention has been directed, very properly, to reform in design of pasteurization equipment and to efficient inspection of plant operation, but this development has been attended by a peculiar neglect of the possible advantages to be derived from intensive laboratory control.

It is well-recognized that a weak link in processing milk may be the handling of the product as it passes from the pasteurizer to the final container. It is here that manual manipulation of piping and apparatus and exposure of the pasteurized product to contamination of insufficiently cleaned and sterilized equipment necessitates constant vigilance on the part of the operator to prevent conversion of the safe heated product to a recontaminated product which may become a vehicle of dangerous infection. Regular inspection of this stage of the processing is indispensable, for it frequently permits detection of defects accidentally developed in equipment and of bad habits in methods of operation.

Inspection alone, however, is not sufficient. It cannot, in routine practice, follow every detail of the cleaning and sterilizing operations, nor can it always be depended upon to detect every defect which may develop even in the best of equipment. Loose joints, fissures in metal linings, breaks in welded seams in which bacteria may lodge and multiply are probably frequently missed in routine inspection. For example, two experiences encountered during the course of the Montreal Milk Epidemic of 1927 may be recounted. Upon the occurrence of the second outbreak, or recrudescence, of the epidemic the Director of the Provincial Bureau of Health assumed the control of the milk plant held to be responsible, with one of the writers in charge of operation. Although every effort was being made to locate every possible defect in the equipment, it was not until several days of this control that a serious defect in the cooler was discovered. The soldered seam joining the metal sheeting a few inches above the bottom on the inside face of each curtain of the cooler buckled open slightly over a distance of several feet, whenever the curtain was moved. Samples taken from the pockets thus formed contained as many as 93 million bacteria and 100,000 coli per cc. It was the custom of the foreman of the pasteurizing floor frequently to grasp the end of a curtain and pull it back in order to detect freezing of the milk on the coils. At every such movement of the curtain material was expressed from the open seam to be washed into the cooler basin. When it is considered that the foreman appointed to the pasteurizing floor a short time previous to the typhoid outbreak was found to be a typhoid carrier, that material from his hands was certain to be transferred to the inner face of the curtain to be washed over the open seam, the potential danger of this defect is manifest. If coli and other bacteria were multiplying in these pockets, it is probable that the typhoid bacillus also could multiply there. Steaming of the cooler, as ordinarily practiced, did not sterilize the pockets, for they were protected on the outside face of the curtains by the mass of metal in the angle iron of the bottom. Both steam and hypochlorite were employed freely at the time, the hypochlorite solution being passed through the whole equipment just ahead of the milk. Yet incubation of the bacteria in these pockets was proceeding apace.

Later, another defect was found. By this time routine laboratory control had been established, with coli-aerogenes tests included. The final product which was usually free from coli in 1 cc. quantities began to show the presence of coli in 1 cc. and occasionally in 0.1 cc. quantities. The plate counts, however, were normal. The cooler was again studied carefully, and it was finally found that, upon exerting pressure against one of the standards supporting the refrigerating coils, it rocked slightly upon its base. Another broken joint was revealed from which a small amount of material exuded whenever the standard was rocked by manipulation of the heavy metal curtains. Milk from the coils was continually flowing over this break, yet a pipette held at the joint collected a mixture of milk and material from the joint which contained 1,650,000 bacteria and 10,000 coli per cc. This mixture was being washed continually into the cooler basin. Immediately after the defect was remedied, coli disappeared from the final product.

One point is to be noted in connection with defects of this type: the washing powders used for cleansing the equipment and the attempts to sterilize the equipment may so alter the conditions as to induce variations in the predominating flora. Thus, in one of the open curtain seams described above, the contents on one day contained about 9 million bacteria and 100,000 coli per cc., the next day 93 million bacteria but no coli in 1 cc. As soon as the defect was discovered the curtains were moved back from the coils, so that the milk which might reach them could not flow into the cooler basin. They had been washed, partially steamed and treated with hypochlorite each day, and it was probably these operations which effected the remarkable variation in the flora of the liquid contents.

Recontamination of heated milk due to defects of the general character of those described is particularly dangerous because of its constant operation; it is only a question of time until the chance disease organism may become established, multiply and infect the product.

Recontamination due to insufficient cleaning and sterilization of equipment is an experience common to all who have had to do with control of pasteurization. Such recontamination is usually only intermittent in character, but is nevertheless potentially dangerous, because of the possibility of incubation of disease bacteria, derived from extraneous sources such as carriers, flies and other insects or from insufficiently heated milk, in the material left on the equipment.

In view of the menace presented by recontamination of pasteurized milk or cream it is rather surprising that so little effort has been made in recent years to exploit the possibilities of detection of such failure by laboratory means. The present Standard Methods of Examination of Milk published by the A. P. H. A. provides, as the only defense against this danger, the official plate count. Yet it must be obvious that, because of normal variations in the bacterial content of the final product due to several causes, such as variation in bacterial content and flora of the raw milk, and in the character of the processing, the residual bacterial count of some hundreds or thousands must be increased by further hundreds or thousands before the difference in comparative plate counts assumes significance. This means that large numbers of bacteria may be added to efficiently pasteurized milk through recontamination without any significant effect upon the plate count. Thus, while comparative plate counts may, at times, indicate gross recontamination, supplementary laboratory methods of greater sensitivity than that of the plate count are much to be desired.

THE COLI-AEROGENES DETERMINATION AS A SUPPLEMENTARY METHOD

The coli-aerogenes determination was first officially suggested in the Report of Committee on Milk Supply published by the American Public Health Association in 1920. That report laid down a rather complete program for control of pasteurization by means of this test, requiring absence of coli from 1 cc. portions during the processing. The suggestion apparently was not followed by the study and experimentation with the coli determination which reasonably might have been anticipated. During the past ten years little official notice has been given to this application of the coli test. Possibly the lack of a simple medium to replace lactose broth, which is notoriously unsuited to detection of coli in milk, was partly responsible for this indifference. Possibly undue emphasis on the fact that certain strains of the coli-aerogenes group may survive pasteurization temperatures served further to paralyze initiative in this direction. In any event, it was not until 1927 that Swenarton (1) following a study of samples of pasteurized milk collected from bottling machines and the final container at plants in Baltimore, again seriously broached the subject of the coli determination as a control test, suggesting a maximum limit of 20 per cent of 0.1 cc. portions positive, and proposing a new medium, Gentian Violet Lactose Bile, to be used for the test.

When, following the Montreal Milk Epidemic of 1927, the Quebec Provincial Government conferred upon the Provincial Bureau of Health full control of pasteurization of milk and cream, a thorough investigation of the plants within the Province was instituted, one of us being detailed for this study. As the coli-aerogenes determination had been, for years, with the exception of a short interval, a routine feature of milk examination by the Provincial Laboratories, it was included in this investigation. The findings, as regards the applicability of this test to pasteurization control, were interesting.

Before proceeding to a detailed discussion of the results obtained, the laboratory method employed may be described. As lactose broth is not satisfactory for detection of coli-aerogenes in milk, Kessler and Swenarton (2) suggested the use of a special medium devised by them, Gentian Violet Bile, and showed that it was a reliable medium for the purpose. The Laboratories of the Provincial Bureau had employed the Brilliant Green Bile of Muer and Harris (3) and later had adopted the two per cent Brilliant Green Bile (4) proposed for water examination. To obtain a conception of the comparative value of the two media a series of 139 samples of bottled pasteurized milk was examined by means of both the Gentian Violet Bile and the two per cent Brilliant Green Bile (Digestive Ferments Company dehydrated products). The test involved the placing of 1 cc. of the undiluted milk in each of two Dunham fermentation-tubes; 2 drops (roughly 0.1 cc.) in each of two similar tubes; 1 cc. of the 1–100 dilution in the next two tubes; and finally, 2 drops of this dilution (roughly 0.001 cc.) in the last two tubes. The eight tubes were held together, as a unit, in a zinc box provided with a cover in which a strip of "eiderdown" served as a common cotton plug for all the tubes, the apparatus being identical with that described by one of us (5) for water examination. The appearance of gas after 48 hours on incubation at 37° C. was considered positive. The results of this comparative examination follow:—

	GENTIAN VIOLET BILE	BRILLIANT GREEN BILE
Number of samples examined	139	139
Identical negative results	84	84
Identical positive results	16	16
Results indicating more coli with one than with the other medium	18	21

The two sets of results agree very well. The results with the Brilliant Green Bile were somewhat more satisfactory in that anomalies, with positives in higher dilutions and negatives in lower dilutions, were less frequently encountered with this medium. In consequence, the Brilliant Green Bile (two per cent) was employed, by the method detailed above, in the work to be described.

RESULTS FROM PLANTS OUTSIDE THE CITY OF MONTREAL

Passing now to the results obtained from the investigation of pasteurization plants in the cities and towns of the Province, exclusive of the City of Montreal, it should be noted that this was the first detailed study of these plants which had been attempted and that, in consequence, the samples collected represented operation under practically uncontrolled conditions. The results obtained from the total of 40 plants are shown in table 1. The temperature of pasteurization observed in these plants ranged from 140° F. upward. About three-fourths of the plants were employing temperatures of 143–145° F. The holding period was in all cases 30 minutes.

The striking feature of these results is the absence of coli-aerogenes from the milk after it had been heated. In only two cases were organisms of this group present in one of the two 1 cc. portions examined; all the other results were negative. Apparently in none of these plants were heat-resistant coli a problem, at least at the time of this study. The plate counts of samples of the heated milk also were quite satisfactory. Thus, even under such uncontrolled conditions, these 40 plants were encountering no difficulty in obtaining reasonable plate counts and practical absence of coli-aerogenes from 1 cc. samples of the heated milk.

When the results from samples collected at further stages of the processing are considered, the picture is quite different. The proportion of 1 cc. samples containing coli increased from 3 per cent at the holder to 23 per cent at the cooler, to 42 per cent at the bottling machine, and to 36 per cent in the final container. Table 1 shows that these plants were practically all successful in their attempts to pasteurize the milk, but about half of them were recontaminating the heated milk in the course of the subsequent processing. In some cases the recontamination was indicated only by the plate count, but in others only by the coli-aerogenes determination.

Particular attention is invited to the consistency of the coli results as compared with the plate counts: the latter may vary greatly but the coli figures are usually maintained from stage to stage. The striking significance of the coli results, where positive, is also in marked contrast to the uncertain significance of many of the differences in plate counts. This is not surprising, for the plate count of the milk as it leaves the pasteurizer is, as a rule, a function of the raw milk count which may vary markedly

TABLE 1

Plate counts and presence of coli-aerogenes organisms in raw and pasteurized milk from small plants

PLATE COUNTS					COLI-AEROGENES*				
Raw	Pasteurized	Cooled	Bottler	Bottled	Raw	Pasteurized	Cooled	Bottler	Bottled
110,000	400	1,000	1,200	1,000	222-	——	211-	22—	22—
480,000	2,500	5,000	8,000	3,000	2221	——	21—	2—	2—
310,000	200	800	600	800	2222	——	1—	2221	2222
680,000	9,800	1,200	4,000	1,400	2222	——	——	221-	21—
270,000	18,000	15,000	7,800	7,000	22—	——	——	222-	2222
88,000	45,000	10,000	12,000	9,000	221-	——	1—	2—	21—
14,000	800	400	1,600	1,200	221-	——	2—	2—	1—
61,000	8,000	4,800	2,200	1,800	11—	——	——	2—	——
230,000	4,800	2,800	9,600	6,600	——	——	——	1—	22—
49,000	2,200	6,000	1,600	2,000	111-	——	2—	221-	22—
	3,200	7,600	63,000	23,000		——	222-	2211	2221
560,000	200	400	25,000	7,800	2212	——	2—	221-	221-
6,000,000	26,000	26,000	43,000	120,000	2222	1—	221-	222-	2221
1,400,000	6,800	13,000	53,000	25,000	2222	——	1—	221-	2211
6,000,000	13,000		62,000	22,000	2222	1—		2222	221-
14,000	200		3,200	800	——	——	——	2—	——
1,300,000	400	200	27,000	40,000	2222	All results negative			
110,000	2,600	3,800	14,000	4,800	221-				
75,000	3,000	1,000	17,000	13,000	2—				
140,000	6,600	13,000	34,000	32,000	221-				
18,000	1,000	400	48,000	36,000	2221				
530,000	4,200	200,000	19,000	17,000	221-				
140,000	200	200	18,000	200	2—				
1,200,000	1,600	2,400	6,000	3,200	221-				
130,000	400	600	2,000	600	21—				
220,000	1,200	500	1,000	2,500	22—				
8,200	200	200	600	200	11—				

* Coli-aerogenes:—2 portions examined of each of the following quantities:—1 cc., 0.1 cc., 0.01 cc., 0.001 cc.

The results are shown in this order. Thus, 21— signifies that coli-aerogenes organisms were found in both of the 1 cc. portions, in one of the 0.1 cc. portions and in none of the other portions.

The table includes results only from those plants where a sample at a stage subsequent to pasteurization gave a plate count greater than twice the count of the pasteurized milk, or positive coli results in at least two portions in excess of the number of portions of the pasteurized milk found positive.

as milks from different cans and from different producers are sent through the plant. In consequence, unless the sampler can be certain that he has followed the same batch of milk, not mixed with milk from previous runs, the significance of variation in the milk count from stage to stage in the processing is difficult to evaluate. Further, a difference of hundreds or thousands in counts may be well within the experimental error of results obtained from the usual method of obtaining such counts. On the other hand, the coli content of the milk as it leaves the pasteurizer is usually very low, and the addition of even a few coli during the subsequent processing can be readily detected.

From a consideration of this limited series of results the following observations are pertinent:—

Coli-aerogenes organisms were practically absent from 1 cc. quantities of the pasteurized milk.

Both coli-aerogenes determination and plate counts were necessary for the detection of recontamination.

Of the two methods the coli-aerogenes determination yielded the sharper, and therefore the more significant, as well as the more consistent results in those cases where the organisms were found.

RESULTS FROM MONTREAL CITY PLANTS

Considering now the larger plants supplying pasteurized milk to the City of Montreal, it must be remembered that the conditions under which they operate are rather different from those under which the smaller plants operate, in that the larger plants are usually adequately staffed, well equipped and favored with managements which are usually familiar with the principles involved in the production of safe pasteurized milk. As a consequence, these plants might be expected to turn out a product somewhat superior to that of the smaller plants.

It happens that two series of results of examination of samples of bottled pasteurized milk, picked up from delivery wagons of eight of the largest Montreal plants, are available for comparison. One series was obtained from 96 samples collected between the dates of March 14 and May 27, 1927, during the first stage of the Montreal Milk Epidemic, and examined by the Provincial Laboratories. These samples were taken at a time when the city was in the throes of an unprecedented outbreak of typhoid suspected to be due to infected milk. In consequence, the managements of these eight plants were exercising particular care to distribute a safe product and, so far as they could determine, were taking no chances.

The other series was obtained from 114 samples collected from wagons of these same eight plants in cities and towns adjacent to Montreal during the first six months of 1931.

The coli-aerogenes results obtained from the two series of samples are shown in table 2. (Two portions of each dilution of each sample were tested for gas formation in Brilliant Green Bile.)

TABLE 2

Plate counts and presence of coli-aerogenes organisms in raw and pasteurized milk from large plants

PLANT	NUMBER OF SAMPLES TESTED		PERCENTAGE OF 1 CC. PORTIONS CONTAINING COLI		PERCENTAGE OF 0.1 CC. PORTIONS CONTAINING COLI	
	1927–1931		1927–1931		1927–1931	
A	8	9	0	0	0	0
B	9	5	11	0	11	0
C	15	24	13	4	0	0
D	10	10	35	0	5	0
E	8	7	19	7	0	0
F	16	24	53	4	25	2
G	15	25	70	16	23	0
H	15	10	87	10	77	5

The milk regulations of the City of Montreal, throughout this time, have required a pasteurization temperature of 142 to 145° F. and a holding period of 30 minutes. There is reason to believe that most of the milk, represented by these samples, was subjected to temperatures approaching the upper of these limits.

It will be noted from table 2 that despite the peculiar circumstances obtaining at the time when the 1927 series of samples was collected, coli-aerogenes organisms were very frequently present in both 1 cc. and 0.1 cc. portions examined. All but one of these plants approached Swenarton's criterion of not over 20 per cent of 0.1 cc. samples positive.

The marked improvement in the results obtained from the 1931 series was due partly to improvement in equipment and partly to the efforts of the Division of Food Inspection of the City Department of Health. This division has waged a determined campaign to eliminate recontamination and, convinced of the value of the coli-aerogenes determination, has predicated its efforts largely upon the results obtained from this method of laboratory examination. As a consequence, whereas the percentage of plate counts of over 60,000 was reduced from 11 to 4, the percentage of 1 cc. portions containing coli was reduced from 42 to 6½, and the percentage of 0.1 cc. portions containing coli from 21 to less than 1.

These results show what can be done,—they show that coli can be practically eliminated from 1 cc. portions of bottled pasteurized milk as delivered to the consumer, that only one of these plants failed to reduce the coli content to a limit of 20 per 100 cc. of samples instead of the 200 per 100 cc. of Swenarton's suggested limit. The milk from three of these plants contained no coli in 1 cc. upon examination of a total of 48 such samples from these plants.

CONCLUSIONS

From a consideration of these two series of results from large as well as from small plants, the following conclusions appear to be warranted:—

1. The plate count, as a laboratory method of pasteurization control, is not adequate. Supplementary laboratory methods are much to be desired.

2. Since organisms of the coli-aerogenes group are seldom found, as indicated by gas formation from 1 cc. quantities, in properly pasteurized milk; since the addition of even small numbers of coli added to such milk can be readily detected, and since coli readily become established and multiply in traces of milk in the more dangerous types of defects and on unsterilized surfaces of pasteurization equipment, the coli-aerogenes determination constitutes a reliable supplementary laboratory method of control.

3. Large city plants, and probably small plants as well, can deliver, to the consumer, milk which contains coli in not more than 10 to 20 per cent of the 1 cc. portions tested.

It should be noted that these conclusions are quite in accord with those indicated in the Report of the Committee on Milk Supply of 1919 to which reference has been made.

In view of the potential danger in recontamination of pasteurized milk and the inadequacy of inspection and the plate count to indicate every instance of recontamination it would appear desirable that, pending a search for other supplementary tests, Standard Methods of Milk Analysis include the coli-aerogenes determination as a supplementary method for collecting additional data on the condition of pasteurized milk.

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THE HEAT COAGULATION OF MILK

1.—VARIATIONS IN THE COMPOSITIONS, HEAT STABILITY, AND OTHER TESTS OF MILKS FROM FOUR COWS DURING THE COURSE OF A LACTATION PERIOD

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A knowledge of the factors that are responsible for the variations in the stability of milks to heat is of prime importance in the evaporated milk industry. At present the manufacturer is handicapped by the lack of information concerning the normal variations that may be expected. He is further handicapped by the lack of reliable tests whereby he can foretell the stability of an evaporated product from the behavior of the fresh milk from which it was manufactured. None of the various "rapid" tests which are at present in use—the alcohol test (1), (2), the acidity test (3), (4), the phosphate test (5)—has been found reliable for predicting the heat stability of all samples of evaporated milk.

The "salt balance" of milk, or the ratio of the sum of the gram equivalents of CaO and MgO to the sum of the gram equivalents of citric acid and P_2O_5, has been advanced by Sommer and Hart (6) as being of prime importance in determining its heat stability. These investigators believe (7) that the "salt balance" relationship can be most clearly demonstrated by very small additions of the appropriate salts and that such a procedure is more reliable than a determination of the salt balance by analytical methods as was done in their early work and also by Rogers, Deysher and Evans (8).

Sommer and Hart (9) (7) from their work on fresh whole milk found that "the demonstration that the milk salts have a decided effect upon the stability of the casein in fresh milk is quite conclusive" and further that "this logically leads to the conclusion that they must have a similar effect on the evaporated milk for we are dealing here with a system that is entirely similar, modified only to the extent to which it has been concentrated and the heat treatment and other manipulations it has undergone. This was readily demonstrated by the addition of these salts to concentrated milk both on an experimental scale and under commercial conditions."

This position is not substantiated in later work reported by these authors (7). They state that "with evaporated milk no case has been found where the coagulation was due to a lack of calcium. Even milk samples that in the unconcentrated form showed coagulation in the heat test, on account

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of lack of calcium, when concentrated as for evaporated milk, evidence no such lack of calcium."

The purpose of this part of the investigation has been to determine to what extent compositions, different heat stability tests and other tests commonly employed, of milk from four cows throughout a lactation period, correlate with time of coagulation by heat of both the fresh and evaporated products.

EXPERIMENTAL

Four cows were selected from the herd of the Bureau of Dairy Industry at Beltsville, Maryland. The cows all calved in October, 1928, within a ten-day period. Cows Nos. 811, 813 and 815 were Holsteins, while cow 659 was a Jersey. The feed of the cows, during the period that the milk was examined, was the same and consisted only of grain, hay, silage, and beet pulp. The cows did not receive pasture at any time during the period of November 1, 1928, to November 1, 1929, with the exception that Cow 813 was turned on pasture October 26, 1929.

Twenty-four hour samples of the milk from each cow were examined within four hours after the morning milking. Tests and analyses of the milk were made each month throughout the lactation period, except during the months of June and July.

The tests and analyses which were conducted upon the samples of milks were the following: total solids, fat, ash, calcium, magnesium, phosphorus, citric acid, titratable acidity, H-ion concentration (pH), alcohol, phosphate, buffer intensities and the time of coagulation of the raw milks by rennet and by heat and of their evaporated samples by heat.

The total solids were determined by drying a sample at 100° C. and weighing the dry matter. Fat percentages were obtained by the Babcock test, and the solids-not-fat percentages represent the difference between the fat and total solids.

The titratable acidity is expressed as percentage lactic acid, the titration having been made with N/10 NaOH, with phenolphthalein as the indicator. The H-ion concentration is expressed as pH and was determined potentiometrically with a quinhydrone electrode.

The alcohol test was carried out with 68 per cent alcohol by volume. The test was considered as positive or negative depending upon the presence or absence of coagulation when 2 cc. of alcohol was added to 2 cc. of milk. The phosphate test (5) is expressed as 10 × the number of cc. of M/5 KH_2PO_4 required to coagulate 10 cc. of fresh milk, after the mixture has been placed in a test-tube and immersed in boiling water for five minutes.

Coagulation tests were made upon the unevaporated milk by the use of rennet and by heat, and upon the evaporated milk by heat. Powdered rennet of a strength of 1: 30,000 (Eimer and Amend) was made up into fresh solutions for each series of monthly tests by dissolving 0.5 gr. of rennet in 200 cc. of distilled water. Five cc. of rennet solution was added to 230 cc. of the warm milk in a bottle at 37° C. The milk was quickly mixed and placed in a 37° C. water bath. The time of coagulation was determined by observing the time of flow of a fine stream of milk siphoned out of the bottle by means of a capillary tube. The limit of error for the time of rennet coagulation was ± 0.1 minute. The time of heat coagulation of the milk was determined by holding the canned milk in a pilot sterilizer and withdrawing the cans at intervals to note the time required to bring about coagulation. Fifteen minutes were allowed to bring the milk up to the sterilization temperature. The unevaporated milk was held at 130° C. until it coagulated and the evaporated milk, which was forewarmed to 95° C. for 10 minutes before evaporation to 18 per cent solids-not-fat, under 26 inches of vacuum, was sterilized at 118° C.

The salt analyses were made as follows: Calcium and magnesium were determined by McCrudden's method (10). The P_2O_5 was determined gravimetrically as $Mg_2P_2O_7$, according to the method of the Association of Official Agricultural Chemists (11). Citric acid was determined by Beau's titration method (12). Recent investigations in this laboratory indicate that this method as used here yields results which are probably somewhat too high. However, the values given are entirely comparable among themselves and hence are valid for all comparative purposes.

The excess base was calculated according to Sommer and Hart's method (6). The percentages of each salt were converted into gram equivalents as follows:

$$(a)\ \frac{\text{citric acid} \times 100}{192}$$

$$(b)\ \frac{P_2O_5 \times 100}{71} \times \frac{7}{11}$$

$$(c)\ \frac{CaO \times 100}{56}$$

$$(d)\ \frac{MgO \times 100}{40}$$

The excess base figure is obtained by subtracting the gram equivalents of (Citric acid + P_2O_5) from those of (CaO + MgO).

The buffer intensity of each milk was investigated according to the methods used by Whittier (13). The change in pH was determined over the range from the original pH to pH 4.7. The average dB/dpH was calculated over this pH range. The average buffer intensity divided by the percentage of milk-solids-not-fat gives the average buffer intensity per gram and is reported as Av. dB/dpH / % S.N.F.

The results of the tests and analyses conducted upon the milk of the four cows throughout the course of a lactation period are shown in Tables

TABLE 1

Results of tests and analyses of samples of milk from Cow 659 at intervals during the course of a lactation period

Cow 659—Calved Oct. 17, 1928. Last milked Oct. 29, 1929. Total milk production 8,983.6 lbs.—Total butterfat production 482.82 lbs.

TESTS AND ANALYSIS	DEC. 3	DEC. 10	JAN. 14	FEB 11	MAR. 18	APR. 22	MAY 13	AUG. 12	AUG. 19	SEPT. 23	OCT. 14
Total solids %	14.93	14.69	15.38	15.99	15.89	15.69	15.58	15.50	15.36	16.19	16.13
Fat %	4.70	4.55	5.10	5.50	5.40	5.50	5.35	5.20	5.20	6.00	5.80
S.N.F. (by dif.) %	10.23	10.14	10.28	10.49	10.49	10.19	10.23	10.30	10.16	10.19	10.33
Titratable acidity	.240	.277	.225	.245	.240	.220	.220	.180	.160	.170	.170
pH	6.54	6.57	6.52	6.55	6.60	6.50	6.70				
Heat stability tests											
Alcohol test	–	–	–	–	–	–	–	–	–	–	–
Phosphate test	21	21	22	21	22	22	22				
Coagulation time (minutes)											
By rennet 37° C.		13.60	14.05	13.22	13.20	12.90	17.00				
By heat (Unevap.) 130° C.	30+	38	45	31	54	42	50	27	26	38	45
By heat coag. (18% S.N.F.) 118° C.	18	42	25	16	15	18	17	17	25	22	34
Salt analyses											
Ash %	.78	.76	.74	.785	.77	.749	.755		.78	.746	.746
CaO %	.204	.198	.198	.202	.196	.186	.186		.185	.189	.187
MgO %	.025	.024	.023	.025	.025	.027	.025		.027	.026	.025
P_2O_5 %	.282	.267	.270	.284	.283	.265	.269		.268	.266	.268
Citric acid %	.280	.290	.251	.264	.22	.24	.25		.22	.22	.205
Excess base											
Gram equivalent of (Ca + MgO) − (Citric acid + P_2O_5)	.27	.23	.38	.30	.43	.36	.23		.42	.47	.49
Buffer capacity = dB/dpH from riginal pH to pH 4. 7											
Av. $\frac{dB}{dpH}$	.0275	.0271	.0274	.0277	.0286	.0280	.0254				
Av. $\frac{dB}{dpH}$/% S.N.F.	.00269	.00267	.00266	.00264	.00273	.00277	.00248				

TABLE 2

Results of tests and analyses of samples of milk from Cow S11 at intervals during the course of a lactation period

Cow 811—Calved Oct. 11, 1928. Last milked Oct. 4, 1929. Total milk production 10,681.6 lbs.—Total butterfat production 359.40 lbs.

TESTS AND ANALYSES	DEC. 3	DEC. 10	JAN. 14	FEB. 11	MAR. 18	APR. 22	MAY 13	AUG. 12	AUG. 19	SEPT. 23
Total solids %	11.71	11.77	12.08	11.81	12.14	11.77	11.73	12.73	13.04	
Fat %	3.00	3.00	2.95	3.00	3.20	2.85	3.10	3.50	3.45	
S.N.F. (by dif.) %	8.71	8.77	9.13	8.81	8.94	8.92	8.63	9.23	9.59	
Titratable acidity	.190	.180	.180	.170	.175	.170	.160	.160	.160	
pH	6.60	6.63	6.60	6.65	6.67	6.63	6.59			
Heat stability tests										
Alcohol test	–	–	–	–	–	–	–			
Phosphate test	18	16	16	16	15	14	13			
Coagulation time (minutes)										
By rennet 37° C.		14.05	15.25	18.24	15.88	15.97	18.20			
By heat (Unevap.) 130° C.	26+	33	32	42	40	46	30	28	20	
By heat coag. (18% S.N.F.) 118° C.	14	40	23	20	40	45	45	43	27	
Salt analysis										
Ash %	.74	.72	.72	.725	.73	.73	.737		.79	.91
CaO %	.160	.155	.158	.159	.157	.159	.154		.201	.233
MgO %	.023	.019	.022	.021	.023	.024	.024		.028	.027
P_2O_5 %	.216	.212	.210	.201	.203	.196	.192		.217	.242
Citric acid %	.28	.26	.28	.285	.26	.26	.260		.27	.19
Excess base										
Gram equivalent of $(CaO + MgO) - (\text{Citric acid} + P_2O_5)$	.04	.00	.03	.07	.20	.31	.28		.84	1.67
Buffer capacity $= dB/dpH$ from original pH to pH 4.7										
Av. $\frac{dB}{dpH}$	.0217	.0212	.0214	.0208	.0214	.0221	.0210			
Av. $\frac{dB}{dpH}$/% S.N.F.	.00249	.00242	.00234	.00236	.00240	.00248	.00235			

TABLE 3

Results of tests and analyses of samples of milk from Cow 813 at intervals during the course of a lactation period

Cow 813—Calved Oct. 21, 1928. (Aborted Nov. 14, 1929.) Last milked July 28, 1930. Total milk production 17,232.5 lbs.
Total butterfat production 588.20 lbs.

TEST AND ANALYSES	DEC. 3	DEC. 10	JAN. 14	FEB. 11	MAR. 18	APR. 22	MAY 13	AUG. 12	AUG. 19	SEPT. 23	OCT. 14	OCT. 28
Total solids %	11.93	12.61	12.30	12.00	12.30	12.12	12.22	11.90	12.01	12.99	13.03	13.45
Fat %	3.00	3.75	3.20	3.00	3.10	3.45	3.55	3.20	3.20	3.50	3.85	4.00
S.N.F. (by dif.) %	8.93	8.86	9.10	9.00	9.20	8.67	8.67	8.70	8.81	9.49	9.18	9.45
Titratable acidity	.195	.195	.185	.185	.205	.160	.160	.150	.150	.160	.170	.140
pH	6.56	6.56	6.59	6.61	6.67	6.70	6.71					6.60
Heat stability tests												
Alcohol test	–	–	–	–	–	–	–	–	–	–	–	–
Phosphate test	15	14	15	15	15	14	14					5
Coagulation time (minutes)												
By rennet 37° C.		11.50	13.25	15.62	15.00	21.40	14.40					
By heat (Unevap.) 130° C.	8	8	7	7	5	35		24	35+	40	50	42
By heat coag. (18% S.N.F.) 118° C.	17	25	15	10	12	15		25	25	18	18	26
Salt analyses												
Ash %	.71	.70	.69	.71	.72	.69	.678		.70	.706	.737	.756
CaO %	.150	.155	.155	.156	.160	.149	.146		.156	.164	.184	
MgO %	.02	.020	.020	.021	.021	.023	.020		.024	.023	.022	
P_2O_5 %	.225	.218	.216	.219	.219	.194	.201		.183	.203	.222	
Citric acid %	.235	.245	.240	.247	.240	.250	.22		.25	.22	.237	
Excess base												
Gram equivalent of (CaO + MgO) – (Citric acid + P_2O_5)	–.06	.04	.08	.06	.17	.19	.16		.45	.54	.61	
Buffer capacity = dB/dpH from original pH to pH 4.7												
Av. $\frac{dB}{dpH}$	.0209	.0204	.0209	.0213	.0220	.0210	.0200					.02587
Av. $\frac{dB}{dpH}$/% S.N.F.	.00234	.00230	.00230	.00237	.00239	.00242	.00231					.00274

TABLE 4

Results of tests and analyses of samples of milk from Cow 815 at intervals during the course of a lactation period

Cow 815—Calved Oct. 17, 1928. Last milked Dec. 20, 1929. Total milk production 21,175.8 lbs.—Total butterfat production 695.27 lbs.

TEST AND ANALYSES	DEC. 3	DEC. 10	JAN. 14	FEB. 11	MAR. 18	APR 22	MAY 13	AUG. 12	AUG. 19	SEPT. 23	OCT. 14	OCT. 28	NOV. 5
Total solids %	11.64	11.69	11.29	11.89	11.88	11.54	11.66	11.60	11.60	12.49	13.02	14.31	13.90
Fat %	3.40	3.50	2.80	3.30	3.20	2.95	3.10	3.00	3.05	3.50	3.80	3.97	4.40
S.N.F. (by dif.) %	8.24	8.19	8.49	8.59	8.68	8.59	8.56	8.60	8.55	8.99	9.22	10.34	9.50
Titratable acidity	.155	.160	.160	.175	.178	.160	.150		.160	.170	.160	.150	.140
pH	6.66	6.65	6.62	6.67	6.72	6.61	6.68					6.72	6.69
Heat stability tests													
Alcohol test	–	–	–	–	–	–	–		–	–	–	+	–
Phosphate test	13	12	13	13	12	11	11					7	8
Coagulation time (minutes)													
By rennet 37° C.		24.15	21.02	19.40	22.70	21.30	26.10						
By heat (Unevap.) 130° C.	4	6	5	5	4	9	5		31+	35	43		31
By heat coag. (18% S.N.F.) 118° C.	12	23	12	9	16	25	19		20	20	22		13
Salt analyses													
Ash %	.70	.70	.70	.69	.72	.72	.72		.72	.73	.763		
CaO %	.135	.134	.147	.144	.152	.147	.151		.148	.165	.176		
MgO %	.020	.018	.021	.020	.021	.025	.023		.023	.024	.025		
P_2O_5 %	.185	.182	.181	.183	.188	.183	.184		.178	.188	.196		
Citric acid %	.270	.261	.260	.267	.280	.277	.26		.26	.25	.261		
Excess base													
Gram equivalent of (CaO + MgO) – (Citric acid + P_2O_5)	–.16	–.15	.18	.04	.09	.16	.27		.26	.56	.65		
Buffer capacity = dB/dpH from original pH to pH 4.7													
Av. $\frac{dB}{dpH}$	.0183	.0199	.0196	.0187	.0201	.0210	.0199					.0288	
Av. $\frac{dB}{dpH}$/% S.N.F.	.00224	.00243	.00220	.00221	.00232	.00244	.00233					.00278	

1, 2, 3, and 4. A study of these tables will show that in many cases there is poor correlation among tests which according to the results of former investigators, should show better agreement. The data reported here have been plotted in many different ways in an attempt to analyze and interpret the results. Where reasonably consistent results have been found, figures have been drawn and are included.

There is a noticeable tendency for the total solids and fat percentages to increase as the lactation period progresses. This is in accordance with the normal trend for the milk of most cows.

The variations in titratable acidity and pH did not show any relation to the variations in heat stability of the milks.

The results of the heat stability tests commonly used did not correlate well with the time of heat coagulation of the milks. All four cows consistently produced alcohol negative milk. The phosphate test showed a tendency to decrease as the lactation period progressed, which would appear to indicate that the milk was becoming less stable to additions of hydrogen or calcium ions, but could perhaps be increased in heat stability by the addition of phosphate or citrate.

Ramsdell, Johnson & Evans (5) have recently been able to show a fair degree of correlation between the phosphate number of a milk and the heat stability of its evaporated product. These investigators worked with composite samples of herd milk, however. In the present investigation the milks of individual cows each showed a characteristic phosphate number which did not vary greatly with variations in the heat stability of each milk.

No relationship was found between the time of rennet coagulation and the time of heat coagulation of the different milks. If the time of rennet coagulation is plotted against the excess base no consistent relationship is observed. However, there is a tendency toward a shortening of the time of rennet coagulation as the calcium content of the milks increases, but this tendency is not very marked. Nor was there a tendency for the time of rennet coagulation to vary regularly with the changes in H-ion concentration of the samples of each milk, although the stabilities of the milks from various cows to rennet seemed to bear an inverse relationship to their characteristic H-ion concentrations.

The time of heat coagulation of the unevaporated and of the evaporated milks have been plotted against the time in the lactation period at which they were produced. A study of Fig. 1 will show that it is difficult to interpret this data in any positive manner. It can not be stated, except in the case of Cow 815, that there is any definite relationship between the time of coagulation of the samples of fresh milks and samples of their evaporated products. In most cases the heat stabilities of the milks tended to increase as the lactation period progressed until a maximum was reached

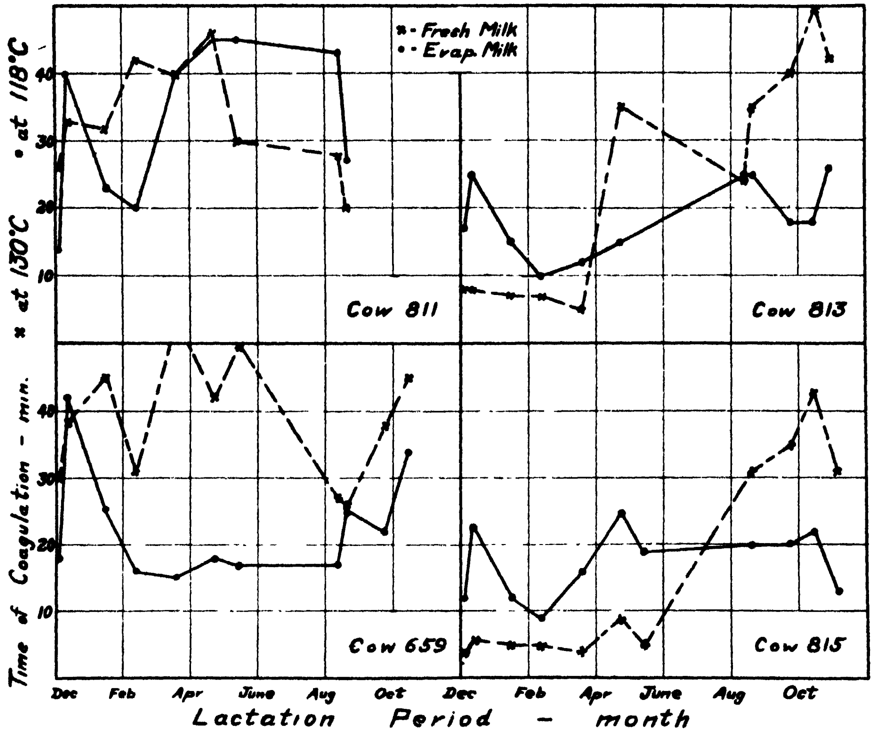

FIG. 1. VARIATIONS IN THE HEAT STABILITIES OF UNEVAPORATED AND EVAPORATED MILKS OF FOUR COWS AT INTERVALS DURING THE COURSE OF A LACTATION PERIOD

in April, when a decrease set in and finally a new maximum in stability was established toward the end of the period, this point being then followed in most cases by a final drop in the time of heat coagulation. Since the feed of the cows remained unchanged during the entire period, these variations can not be ascribed to the effect of green pasture or of other changes in diet.

According to the work of Sommer and Hart (6) there should be a good correlation between the time of heat coagulation of milks and their salt balance. The relationships found between the heat stability and the excess base in the milks examined here are shown in figure 2. The correlation is not good but it can perhaps be said that there is a tendency for the heat stability of these milks to increase as their calcium and magnesium content becomes greater. This is in direct contradiction to the results of Sommer and Hart (6) summarized by their statement, "Those that coagulated had the largest excess of calcium and magnesium."

The effect noted is more pronounced in the case of the fresh milks than in that of the evaporated samples.

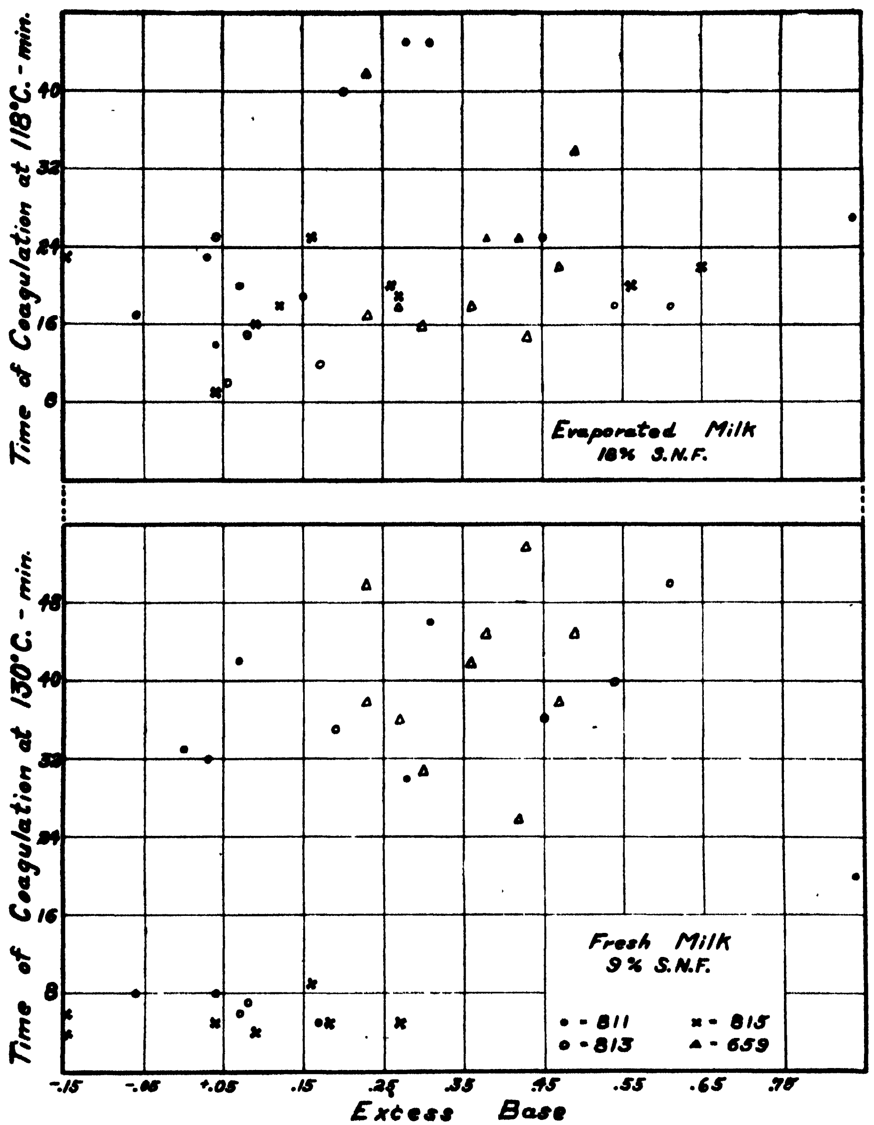

FIG. 2. RELATIONSHIP BETWEEN THE HEAT STABILITY OF UNEVAPORATED AND EVAPORATED MILKS AND THE EXCESS BASE OF THE ORIGINAL MILK

If the excess base is plotted against the time of lactation, a gradual increase is observed as the period progresses. However, a number of irregularities may be noted in such a figure.

Each milk had a characteristic buffer intensity curve which, although it varied to some extent, still retained its individuality when it was compared with the curves of other milks. Only the average buffer intensity

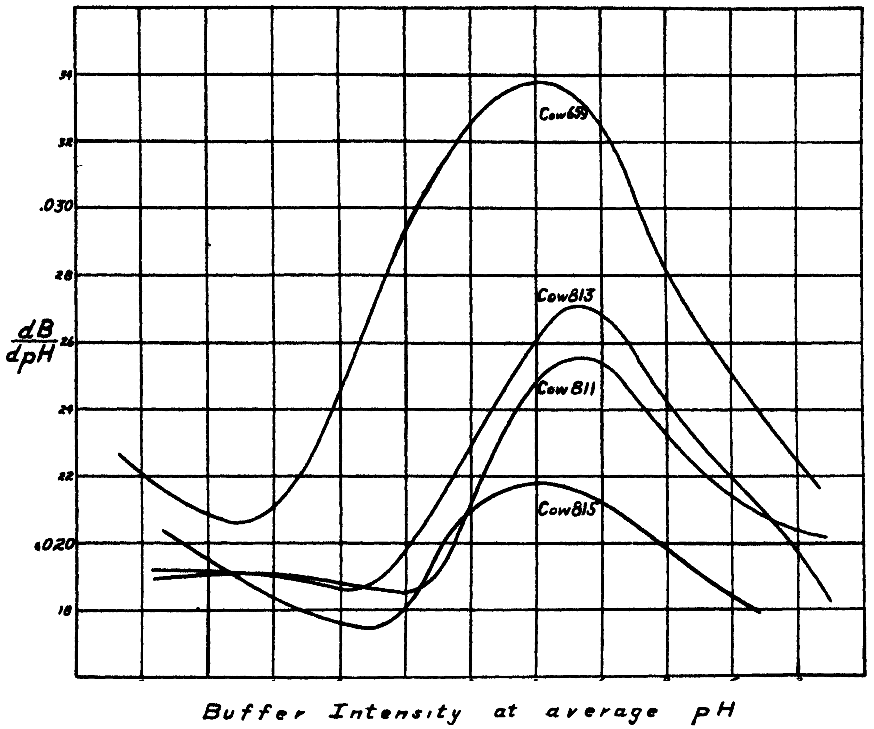

FIG. 3. AVERAGE BUFFER INTENSITIES OF THE FOUR MILKS, OVER A RANGE OF pH OF APPROXIMATELY 4.2–6.2, ON DECEMBER 10

data has been reported but figure 3 shows the buffer intensity curves of the four milks studied, as they were on December 10. There is a marked contrast between that for the milk from Jersey Cow 659 and that for the milk from Holstein Cow 815 in respect to the buffer capacity of their milks. The milk of the Jersey cow is typical of milk secreted by cows of this breed but the milk from Cow 815 is somewhat lower in buffer capacity than is normal Holstein milk. Maximum buffering occurs in each case between pH 5.4–5.6.

When the average buffer capacity of each milk is divided by its percentage of solids-not-fat, the resulting figure, the average buffer intensity per gram of solids, shows uniform variations with advances in the lactation period. From the figure obtained for early December a drop occurs to a minimum point in January or February. A gradual rise in dB/dpH / % S.N.F. now sets in until a maximum is reached in April, at which time a maximum in heat stability occurred also, as was previously shown. At this time there is an abrupt drop·in buffer intensity up to the middle of May, after which time these measurements were discontinued except for two final figures obtained at the end of the period which show the milks probably greatly increase in their buffering as the lactation time draws to a close.

DISCUSSION

The great variations observed in the results obtained with these four milks emphasizes what a wide difference exists in the properties of the milks. It does not seem improbable that the two extremes which would normally be encountered are represented by the milks of cows 659 and 815.

During the first few months of this experiment a composite sample of these four milks was also examined but since it did not show as much consistency in its behavior as did the individual milks, the composite analyses were discontinued.

The lack of correlation in the data presented here is much more striking than the few instances in which some degree of consistency in results has been obtained. The work does not strengthen any existing theory of heat stability but rather it shows that the correlations necessary for the support of any existing theory are lacking.

The early contention of Rogers, Deysher and Evans, as opposed to that of Sommer and Hart, that the heat stability of an evaporated milk bore no relation to the stability of the original fresh milk is confirmed by the data presented here.

That the salt balance theory of Sommer and Hart, while it does hold in some cases, explains only a part of the heat stability phenomena in milk, is conclusively shown by the results of the analyses given above. Many milks with a salt balance favorable to maximum stability as interpreted by them failed entirely to exhibit such a characteristic. Sommer and Hart have pointed out, that the salt balance can be shifted by amounts of salt well within the limit of error of analytical determinations. It does seem possible nevertheless, that such analyses would bear some relation to the heat stability of a milk if there were no other important factor controlling stability than salt balance.

The results of this study of the milks of individual cows serves to further emphasize the complexity of the problem of heat coagulation in milk. While the results are essentially negative in that they do not strengthen any existing views on the subject, yet they have a very positive value. The data show where correlations do not exist.

The conclusions of Benton and Albery (2) that for each milk there is an optimum combination of pH and salt balance which will give maximum stability is further emphasized by these results. "This optimum is probably the resultant of several variables and consequently is an expression of the colloidal peculiarities of the particular sample." Obviously there are other as yet unknown factors influencing the process of coagulation of milk by heat.

SUMMARY

The relationship between the heat stability and the composition and other properties of the milks of four cows during the course of a lactation period was investigated. No marked correlation was found between the heat stability and the various tests and analyses conducted upon the milks. The "rapid" tests sometimes used to classify milks to be used for condensing, such as the acid test, the alcohol test, and the phosphate test, did not satisfactorily indicate the relative heat stability of the milks. The salt balance as determined by analyses of the milks showed no direct correlation with the heat stability of the samples. The buffer intensity was not related to the stability of the milks toward heat. No clear relationship was noted between the heat stabilities of the fresh milks and of their evaporated products. Each of the individual milks for the most part retained fairly constant values in many of the tests, these values being characteristic of the milk from each cow.

The results of this investigation of the individual milks of four cows conclusively demonstrated the inadequacy of our present knowledge of the heat stability of milk.

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THE HEAT COAGULATION OF MILK

II. The Influence of Various Added Salts Upon the Heat Stabilities of Milks of Different Concentrations

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The colloidally dispersed calcium caseinate is the phase principally concerned in the coagulation of milk. It exists in a finely divided state (1) and is equilibrated, with respect to composition and charge, with the electrolytes in the serum in which it is dispersed. The particles are negatively charged and to a certain extent hydrated. To these properties they owe their colloidal stability.

On the basis of the theory of colloid stabilization and destabilization the addition of positively charged electrolytes to milk should tend to precipitate the calcium caseinate particles. It is significant, however, that addition of these electrolytes even in relatively large quantities at room temperatures does not cause precipitation. Addition of salts containing heavily charged negative ions should stabilize the calcium caseinate by further increasing the charge on each particle. But, contrary to the general rule for the stabilization of colloids, some milks, when judged by their resistance to coagulation with heat, are stabilized by addition of positively charged electrolytes while others are stabilized by electrolytes of the opposite character.

This has been shown in studies on heat coagulation by Sommer and Hart (2) and by Benton and Albery (3). Sommer and Hart have expressed the ratio of the amounts of positive ions (Ca and Mg) to that of negative ions (phosphates and citrates) as the "salt balance" (see our previous paper, p. 5) and have maintained that "a proper balance of these two classes of milk constituents will prevent the coagulation of the milk in the heat test; an excess of either one of these two causes the milk to coagulate." No definite meaning can be ascribed to the expression "proper balance" since the ratio is not a constant, and the type of electrolyte which will stabilize a milk as well as the amount is a matter of experimental determination.

The same authors have also concluded that "the salt balance, which was found to affect the coagulation of fresh whole milk at 136° C. also affects the heat coagulation of evaporated milk at the lower temperature used in commercial sterilization," and "The knowledge of salt balance gained by a

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study of fresh milk samples at 136° C. has been successfully applied in preventing troublesome coagulation of evaporated milk."

However, results indicating that the additions of calcium do not produce analogous effects in an unevaporated milk and its evaporated product have also been noted by Sommer and Hart (2). It seems therefore evident that in dealing with the evaporated product wherein phase concentrations have been increased and salt equilibria altered, the reactions to heat are not analogous to those of the original milk. On the basis of the effect noted of various ions upon dilute and concentrated sols, the concentration of a milk should tend to decrease the relative precipitating effectiveness of the di- and trivalent, and increase the effectiveness of the monovalent ions. The effect of forewarming and concentration upon the salt constituents can not be surmised.

The results presented in our previous paper, as well as of those reported by Sommer and Hart and by Benton and Albery, indicated therefore that a more comprehensive and detailed study of the effect of different added electrolytes upon the heat stability of milks of different concentrations was necessary to aid in clarifying the problems.

COAGULATION WITH HEAT IN THE PRESENCE OF ADDED ELECTROLYTES

Preliminary to the work on the effects of electrolytes on coagulation by heat, the relationships of time and temperature of coagulation were determined in order that a temperature might be chosen which would properly reflect stability variations. The relationship of forewarming, a commercial practice, to the stability of samples of a milk of different concentrations was also established in order that the results obtained might be properly interpreted in the light of commercial practice.

In the following work the time of coagulation of the milks was obtained by heating them in sealed tubes immersed in a glycerine bath, at 120° C. unless another temperature has been given. Skimmed milks were used throughout, since it has previously been shown (4) that the presence of small percentages of fat in its natural state of dispersion does not change the heat stability relations involved.

Aqueous salt solutions of different concentrations were made up. The required amount of salt solution, not to exceed 1 cc., was pipetted into a test tube together with enough distilled water to give a total of 1 cc. of aqueous solution, when less than 1 cc. of salt solution was used. To this was added 5 cc. of the milk to be tested, making the total volume in the tube equal to 6 cc.

The Time-Temperature Relationship

The effect of various temperatures from 80° to 120° C. upon the time of coagulation of several milks of 9 per cent and 18 per cent solids-not-fat concentration was determined in the manner described. The results, plotted in Fig. 1, show that as the temperature is increased the time of coagulation decreases logarithmically, although the rate of decrease in the case of milks of 18 per cent solids-not-fat is greater than in those of 9 per cent solids-not-fat concentration.

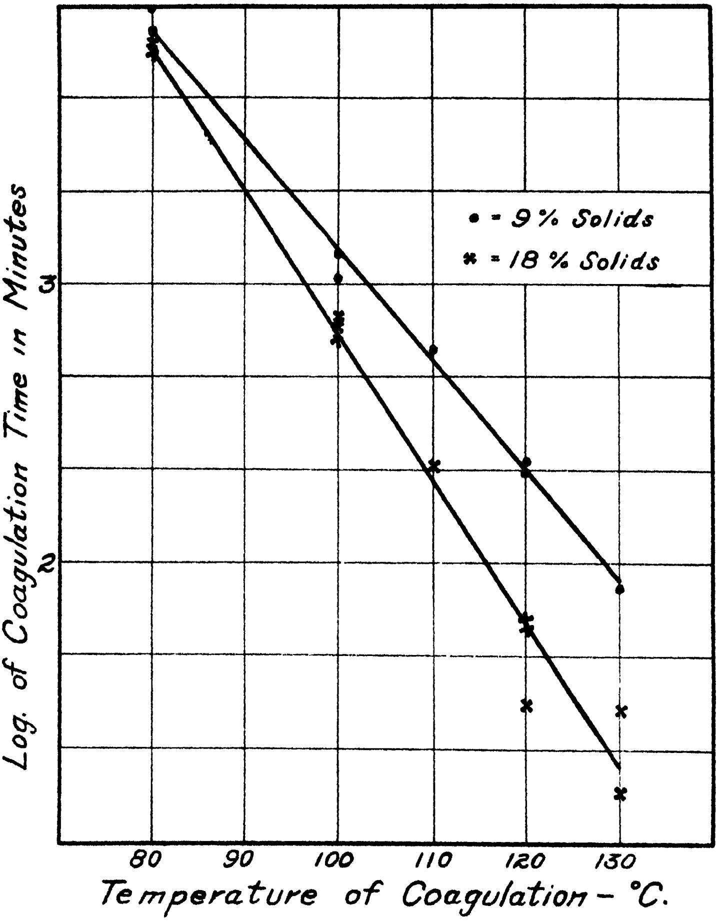

FIG. 1. THE RELATIONSHIP BETWEEN THE TEMPERATURE AND TIME OF COAGULATION OF SKIMMED MILK OF 9 AND 18 PER CENT SOLIDS CONTENT.

The milks used in the above experiments were of good quality and of approximately the same stability to heat. Milks of lower quality would yield results of a lower order of magnitude (4). In these latter cases the results obtained with the samples of 18 per cent solids-not-fat concentration at temperatures of 130° C. or above are somewhat high, due in part to the proportionately large value of the time necessary for the samples to come to the temperature at which they are to be maintained.

The data indicate also that, aside from the practical impossibility of using the lower temperatures, they do not yield results on milks of 9 per cent solids-not-fat concentration whose differences are directly comparable with those obtained on the same milks reduced to 18 per cent solids-not-fat concentration.

In experiments carried out at 115° C. or at lower temperatures, the time element involved is too great to make these conditions practical, especially in the case of skim milks of 9 per cent concentration. Too high temperatures, 130° C. and higher, give results with skim milks of 18 per cent concentration that may be in error as heretofore stated. Hence a temperature of 120° C. was chosen as optimum from the standpoint of experimental conditions.

Forewarming

The temperature of forewarming of a milk evaporation is known to influence the heat stability of its concentrated product. The effect of forewarming changes, however, with a change in the solids-not-fat content of the milk. The magnitude of this change is shown in figure 2. The data in this figure were obtained by forewarming part of a batch of milk to 95° C. for 10 minutes before evaporation. The control sample was not forewarmed. The two milks were then evaporated to the specified concentrations and their heat stabilities were determined. The results show that milk having a solids-not-fat content of 13.5 per cent is not changed in sta-

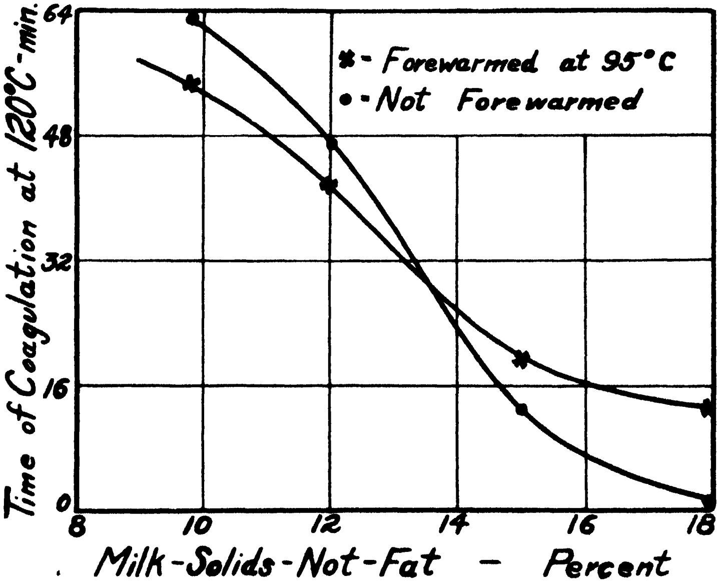

FIG. 2. THE EFFECT OF FOREWARMING UPON THE HEAT STABILITY OF A SKIMMED MILK REDUCED TO VARIOUS CONCENTRATIONS.

bility by forewarming, but in milk below this value forewarming is detrimental and at concentrations above 13.5 per cent stability is improved. This critical solids content was found to range from 10.5 to 14 per cent, depending upon the individual milk used.

A similar relationship is shown in figure 3. In this case the milk used was increased in heat stability by the addition of the proper amount of $AlCl_3$. Excessive amounts of the salt decreased stability. The figure shows the maximum stability which it was possible to attain in the milk by additions of $AlCl_3$. Different percentages of solids were used and each milk was forewarmed to 95° C. and to 70° C. The critical solids content where forewarming does not affect stability is here shown to be 10.5 per cent.

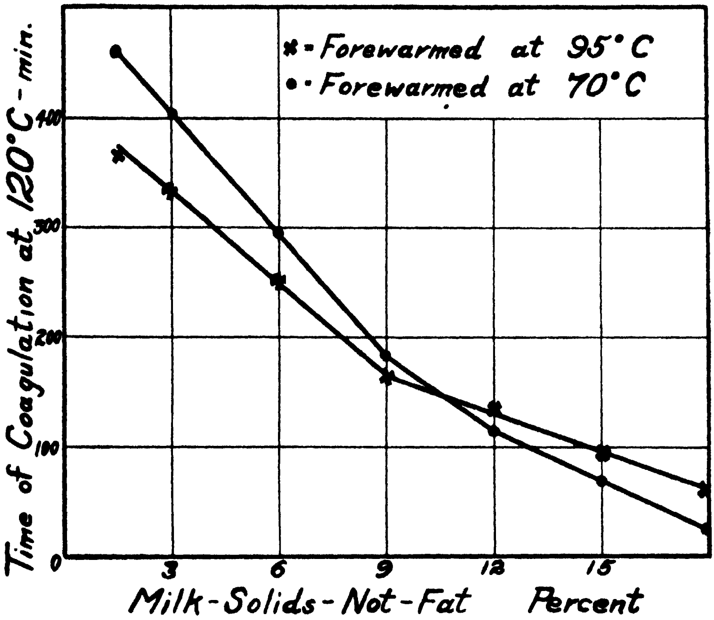

FIG. 3. THE MAXIMUM HEAT STABILITY WHICH WAS ATTAINED BY THE ADDITIONS OF $AlCl_3$ TO SKIMMED MILKS FOREWARMED TO 70° C. AND 95° C., RESPECTIVELY, AND REDUCED TO VARIOUS SOLIDS CONCENTRATION.

The effect of the addition of $CaCl_2$ upon the heat stability of a milk of 18 per cent solids which had been forewarmed to 95° C. and to 65° C. is shown in figure 4. This is a type of milk which is increased in stability by calcium additions and will be discussed later. It is interesting to note that the curves are similar but represent different degrees of stability, which is controlled by the temperature of forewarming.

Figures 3 and 4 show that there is a fundamental change of unknown nature which is brought about by forewarming. This change is not

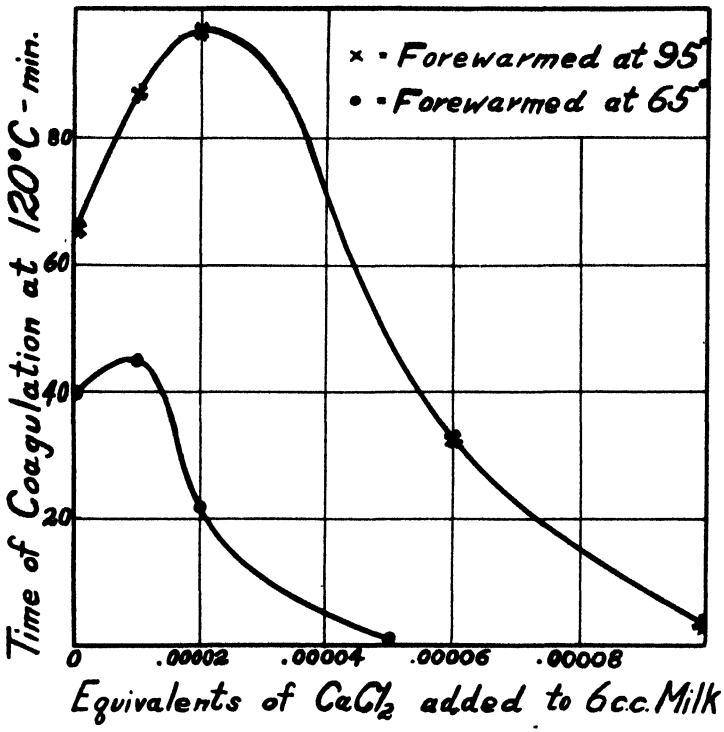

FIG. 4. THE EFFECT OF THE ADDITION OF $CaCl_2$ UPON THE HEAT STABILITY OF SKIMMED MILKS OF 18 PER CENT SOLIDS CONCENTRATION AND FOREWARMED TO 95° AND 65° C.

affected by additions of salts to the milk. The same relationship between milk forewarmed to 95° C. and 65° C., as that shown in figure 4, was obtained when $BaCl_2$, $AlCl_3$, $ThCl_4$, K_2HPO_4, or $K_2C_6H_7O_5$ was used in place of $CaCl_2$. It was found impossible to restore the stabilities obtained for milk forewarmed at 65° C. to the high stability resulting from 95° C. forewarming by the addition of any of the above salts. In no case was the higher temperature curve lowered to coincide with the 65° C. curve when equal quantities of the same salt were added to milks forewarmed to each of these temperatures, respectively.

The results indicate that similar conditions of forewarming must be practiced in all cases where comparisons are to be made and that the addition of electrolytes does not alter the relationship established by forewarming except in degree of stability. It appears that forewarming does not affect stability merely through changes in the equilibria of the soluble salts since the stability cannot be restored by the addition of electrolytes in those cases wherein it has been reduced. Forewarming seems therefore to cause an irreversible change which appears to be corollary to other changes caused by heat. This change may be one of variations in the hydration of the calcium caseinate particle due to alteration in its composition.

Two Types of Milk

It has been shown by previous investigators (2) (3) (5) that two different types of milk exist with respect to the effect of the hydrogen ion concentration of the milk upon its heat stability. It has been demonstrated that the addition or bacterial development of acid in a milk may either increase or decrease its stability. The final effect depends upon securing in the milk the optimum pH for the particular sample in question.

Investigations on the effect of pH upon the heat stability of a milk have not previously included results which directly compared the fresh milk with its concentrated product. In figures 5, 6 and 7 are plotted data obtained by making such a comparison, using different types of milk. In figures 5 and 6 the increase in acidity was produced by the addition of lactic acid to and by the action of bacteria upon the fresh unconcentrated

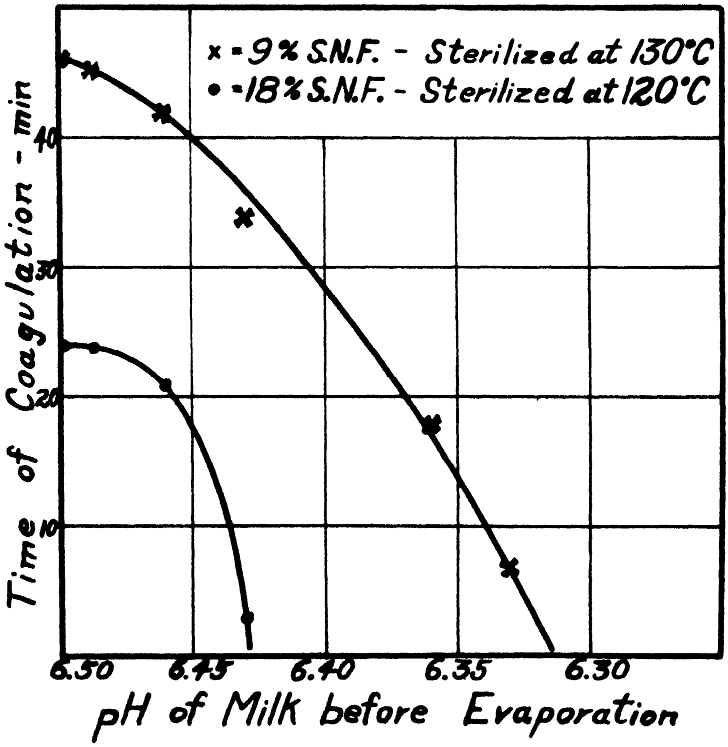

FIG. 5. THE EFFECT OF H⁺ ION CONCENTRATION UPON THE HEAT STABILITY OF ONE TYPE OF FRESH MILK AND OF ITS CONCENTRATED PRODUCT. MILK FOREWARMED TO 95° C. BEFORE EVAPORATION. ACIDITY OBTAINED BY THE ADDITION OF LACTIC ACID TO THE RAW MILK.

skimmed milk, respectively. Half of each sample was then evaporated to 18 per cent solids and the heat stability of all samples determined. The data shown in figure 7 were obtained by the addition of HCl to fresh and concentrated samples of a single milk just before sterilization.

The milk upon which the values shown in figure 6 were obtained is typical of the majority of normal milks. An increase in acidity decreased the stability of both the fresh and concentrated samples. An increase in heat stability was brought about in both the fresh and evaporated milks shown in figure 6 by a small increase in acid. Milk of this type which is not already at its optimum pH to give maximum stability toward heat is frequently encountered. The results shown in figure 7 were obtained from a milk which was different from either of the two preceding types. Addition of HCl to the concentrated sample increased its stability to heat. Obviously in a milk of this type it would be impossible to apply to the concentrated product any results obtained with the initial milk as Sommer and Hart found could be done in their work. Concentration not only lowers stability but alters the effect of acids upon the subsequent stability of the

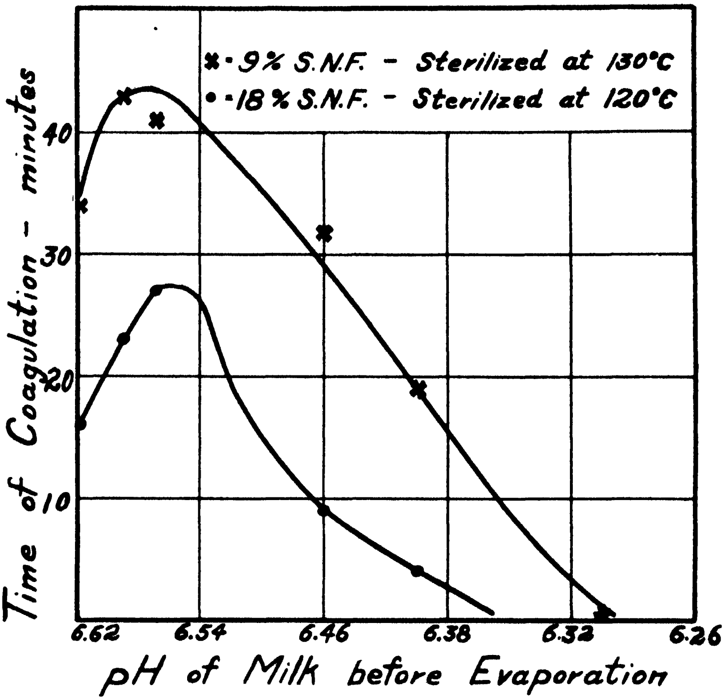

FIG. 6. THE EFFECT OF H^+ ION CONCENTRATION UPON THE HEAT STABILITY OF ONE TYPE OF FRESH MILK AND OF ITS CONCENTRATED PRODUCT. MILK FOREWARMED TO 95° C. BEFORE EVAPORATION. ACIDITY OBTAINED BY THE ACTION OF BACTERIA DURING NORMAL AGING OF MILK.

milk. Two types of milk exist, therefore, with respect to their stability to acids and the degree to which their normal concentrations are affected is not necessarily reflected in their concentrated forms.

Effect of Various Electrolytes Upon Heat Stability

The facts cited question the use of buffer salts to stabilize all milks toward heat. Furthermore, it is known that the hydrogen ion has a high degree of activity in affecting ζ-potential and stability changes of various colloidal dispersions, and the question naturally arises to what extent other ions can promote the same change. This question becomes more important when it is considered that there is a great difference in the magnitude of the action of ions of different valences in various colloid sols.

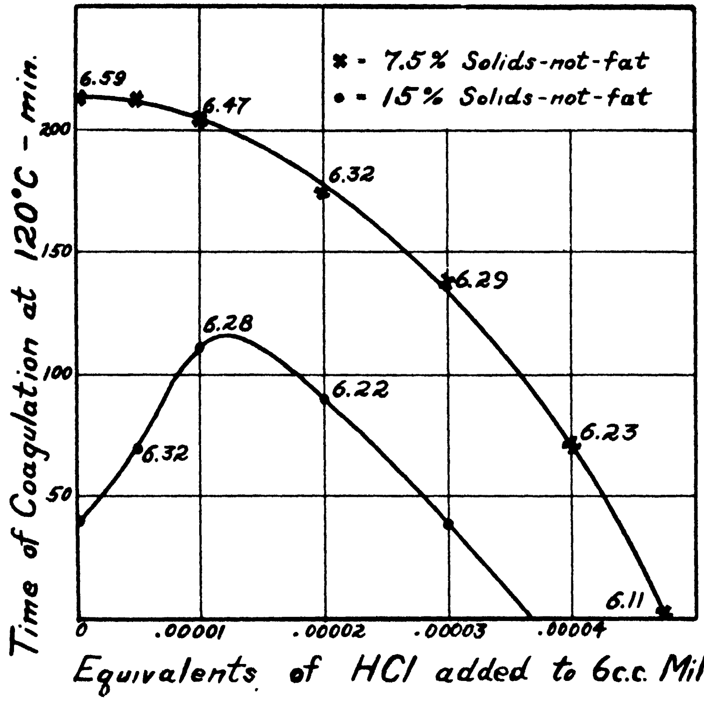

Fig. 7. The Effect of the Addition of HCl Upon the Heat Stability of One Type of Fresh Milk and of its Concentrated Product. Milk Forewarmed to 95° C. Before Evaporation. The pH Values After Each Addition of Acid are Inserted in the Figure.

The quantitative effect of mono-, di-, tri- and quadrivalent ions upon milks of various concentrations was first studied.

The effect of the addition of different electrolytes upon the heat stability of skim milks of different solids content is shown in figures 8 to 14. The salts used were KCl, HCl, $BaCl_2$, $CaCl_2$, $AlCl_3$, and $ThCl_4$. Complete curves were obtained for each salt and for milks of several concentrations. The data extend from no additions of salt to addition of large enough quantities of salt to decrease the stability of the milk to a value of zero minutes at 120° C. The data, given in figures 8 to 14, were obtained on different milks, although each separate figure is plotted from data obtained with a single milk.

Figures 8 to 14 show also that a certain critical salt concentration existed beyond which rapid sensitization of the sol to heat occurred. The value of this critical salt concentration was dependent upon the solids content of the milk and upon the precipitating ion. As the percentage of milk solids in the sol decreased, the fall from maximum stability to immediate coagulation became more abrupt and less salt was required to bring about this sharp drop in stability. The abruptness of the drop when the critical salt concentration was reached appeared to be dependent upon the nature of the precipitating ion. Hydrogen, thorium, and aluminum caused rapid

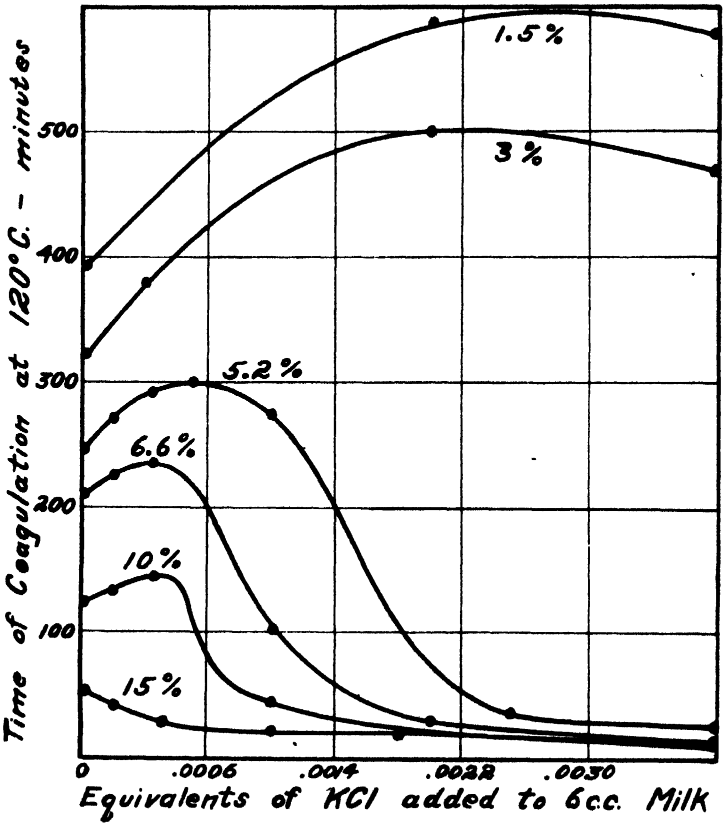

FIG. 8. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF KCl TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION

changes in heat stability after passing the critical salt concentration while potassium, barium and calcium had a milder effect.

The foregoing results show that the milks of lower concentrations are the most sensitive to the effect of tri- and quadrivalent positive ions. The higher concentrations are more sensitive to variations in the amounts of the mono- and divalent positive ions, HCl and Ba and Ca salts being highly effective at all concentrations. The relationship is similar to that determined for As_2S_3 sols with K, Ba and Al ions (6, p. 449). It seems, therefore, that the calcium caseinate suspensions are somewhat analogous to hydrophobic sols in their reactions toward electrolytes.

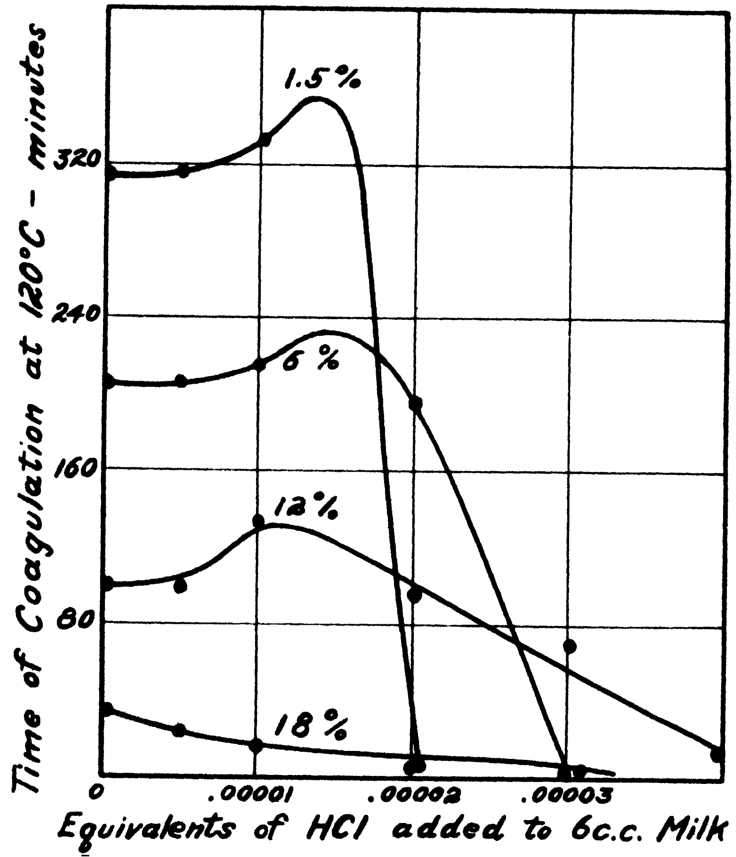

FIG. 9. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF HCl TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

The effect of additions of different quantities of di-potassium acid phosphate and potassium citrate upon the heat stability of a fresh skimmed milk is shown in figure 14. Attempts were also made to adjust the pH of the milk to that of the original sample before the additions of salt had changed it. Two curves, with and without pH adjustments, for both citrate and phosphate additions have been included in figure 14.

The effect of the phosphate and citrate ions upon the stability of fresh milk is not the result of a change in pH. The results given in figure 14 show that when the pH is not adjusted after the salt additions to that of the original milk, the stability is only a little greater than when the pH is lowered by HCl additions.

The abrupt decline in the citrate curves in figure 14 is noteworthy. The same effect, although less marked, would be shown in the phosphate curves if they were extended farther than is shown in the figure. This rapid decrease in heat stability of an unconcentrated milk with very small additions of salt probably indicates that the potential on the particle has reached its critical value. Stability has increased to a maximum at the point where a proper balance in particle charge has been attained. The delicate balance is completely upset by further salt additions.

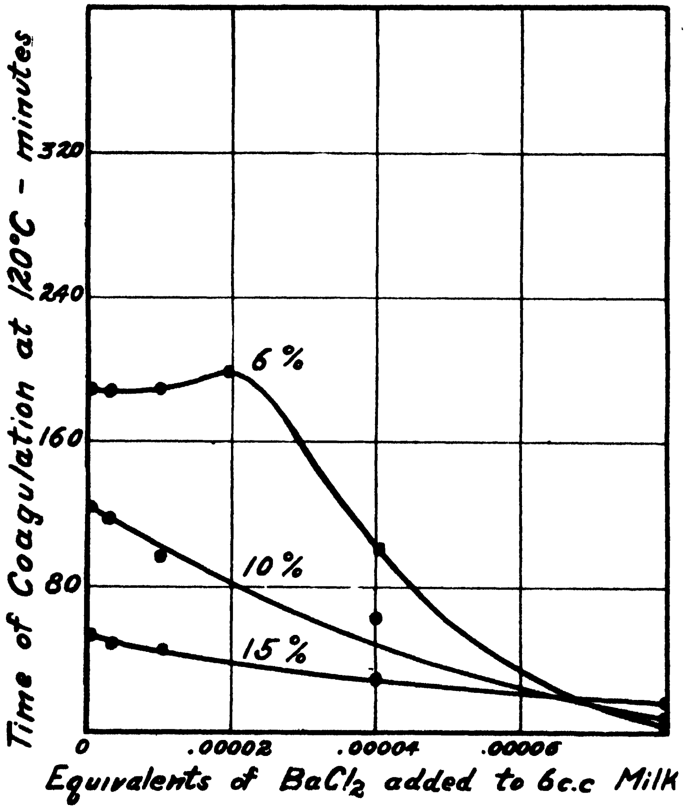

FIG. 10. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF $BaCl_2$ TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

The results in figure 14 show, especially in the case of citrate additions, that the range of maximum stability lies within comparatively narrow limits with respect to salt concentrations.

Sommer and Hart (2) found that citrates produced greater stability than phosphates for a given quantity of added salt. The data of figure 14 is in agreement with this finding. Potassium citrate produces a stronger and more powerful effect upon heat stability than does the phosphate. The maximum stability which can be attained by the addition of these negative ions is not, however, widely different.

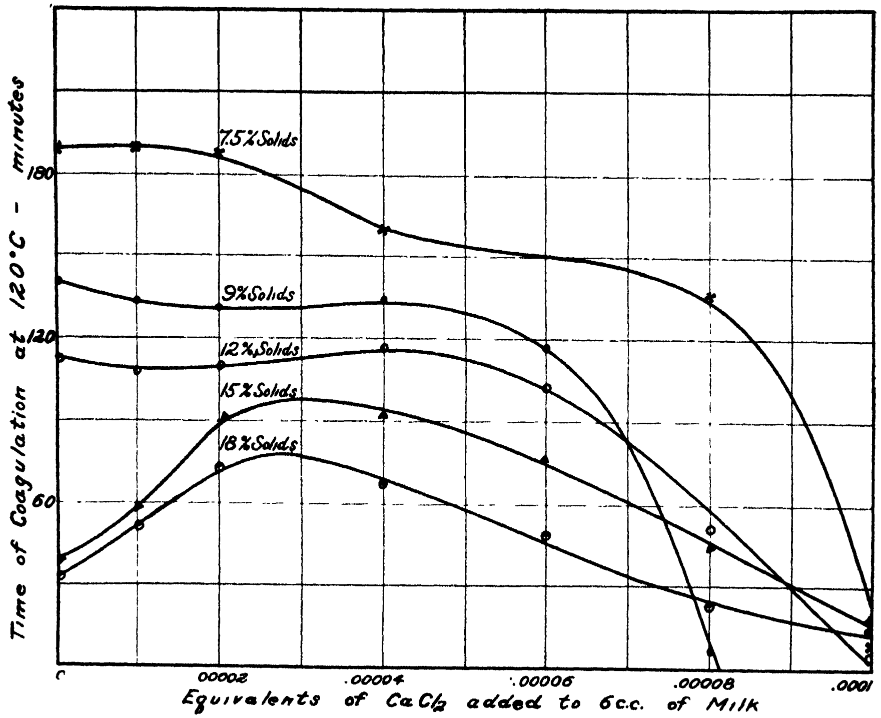

FIG. 11. THE EFFECT UPON HEAT STABILITY OF ADDITIONS OF $CaCl_2$ TO SKIMMED MILKS OF DIFFERENT SOLIDS CONCENTRATION.

It should be mentioned in passing that where no compensation was made for the increase in pH which occurred after large additions of citrate and phosphate to the milks, there was a marked tendency for the calcium caseinate to pass into solution. The first noticeable opalescence occurred when the amount of citrate exceeded 0.0003 and of phosphate 0.001 equivalents per 6 cc. of milk of 7.5 per cent solids after dilution. Reference to figure 14 will show that the curves were practically completed before this quantity of salt was used.

The work previously described has shown that with respect to the effect

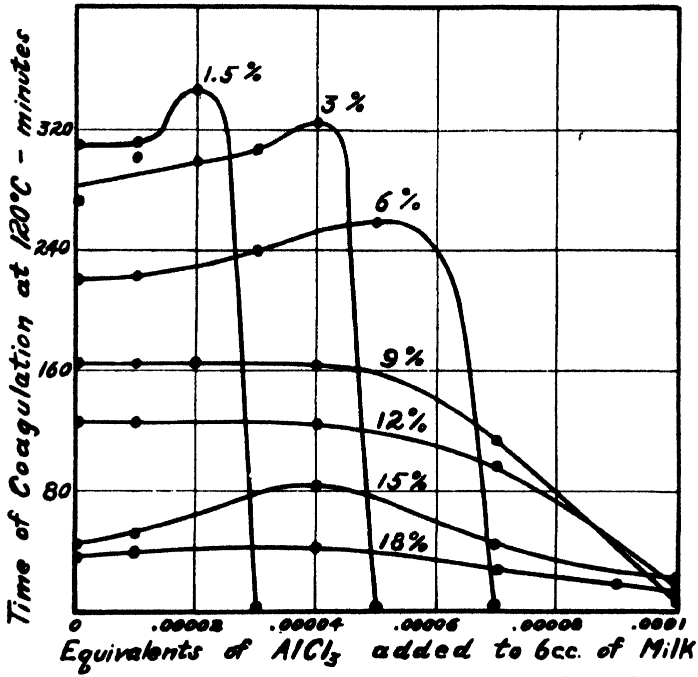

FIG. 12. The Effect Upon Heat Stability of Additions of $AlCl_3$ to Skimmed Milks of Different Solids Concentration.

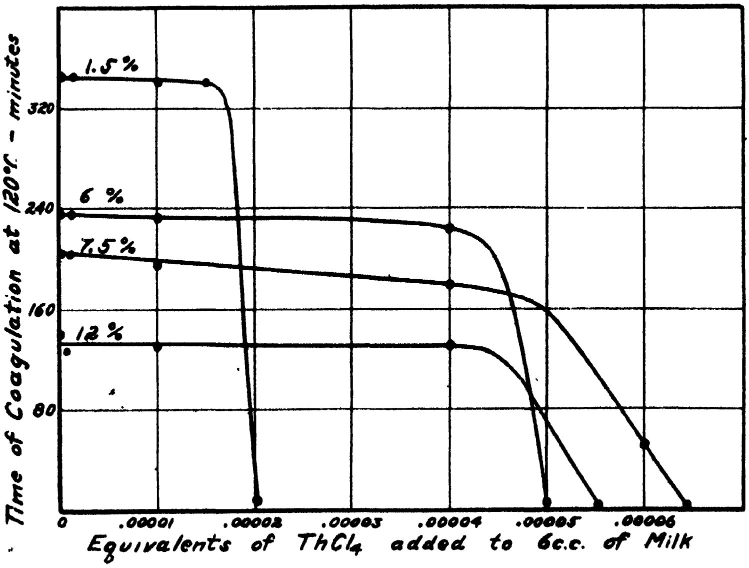

FIG. 13. The Effect Upon Heat Stability of Additions to $ThCl_4$ to Skimmed Milks of Different Solids Concentration.

of various positive and negative ions two types of milk exist. The magnitude of the effects produced has also been shown.

The effect of ions of different valence upon the two types of milks of high solids-not-fat concentrations is shown in figure 15. The milks used were mixed herd milks secured from the same herd on different days. All the samples were forewarmed to 95° C. for 10 minutes before evaporation. The curves which show stabilization by the different cations are plotted from data which were obtained from the same milk sample (milk of Jan. 7, 1931) concentrated to give a final milk solids content of 15 per cent. The lower group of curves in figure 15, in which notable stabilization was obtained with K_2HPO_4, are plotted from milks concentrated to yield a final solids content after dilution with salt solution of 18 per cent. These curves were obtained from milks secured on the following dates:

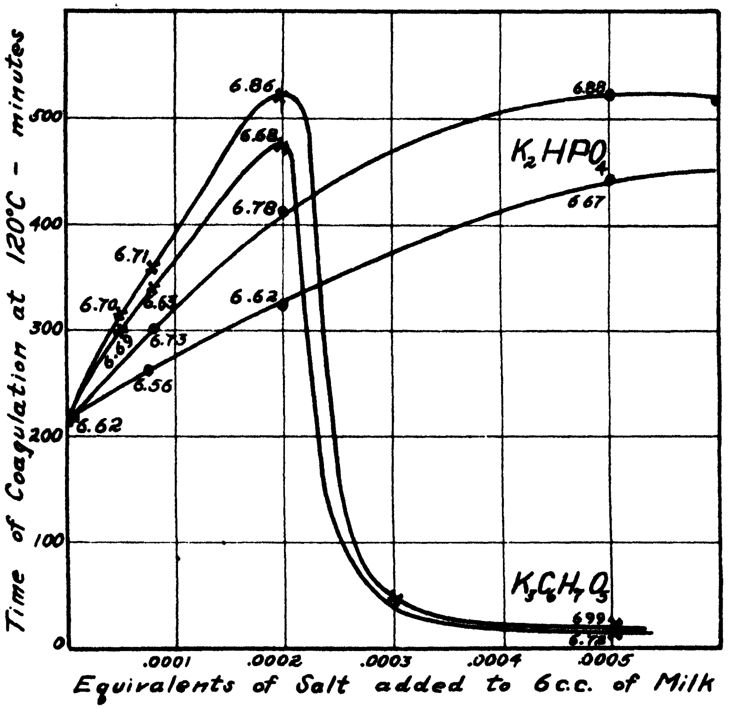

FIG. 14. THE EFFECT OF ADDITIONS OF POTASSIUM PHOSPHATE AND POTASSIUM CITRATE UPON HEAT STABILITY OF PLAIN SKIMMED MILK WHEN THE pH IS AND IS NOT READJUSTED BY ADDITIONS OF HCl AFTER THE SALT ADDITIONS. THE INSERTED FIGURES ARE THE pH VALUES. THE SOLIDS CONTENT OF THE MILKS AFTER ADDITION OF SOLUTIONS WAS 7.5 PER CENT.

1. KCl $BaCl_2$ and $AlCl_3$—Aug. 5, 1930.
2. HCl —Sept. 9, 1930.
3. $CaCl_2$ K_2HPO_4 —Jan. 12, 1931.

Three separate milks are, therefore, concerned in the work shown in this part of the figure.

The fact that one set of curves is from milk of 15 per cent solids and the other, 18 per cent solids, does not lessen the value of the comparison between these two types of milk. The effect would be the same if both milks were standardized to either solids content, except that a small difference in coagulation time would result from the concentration differences.

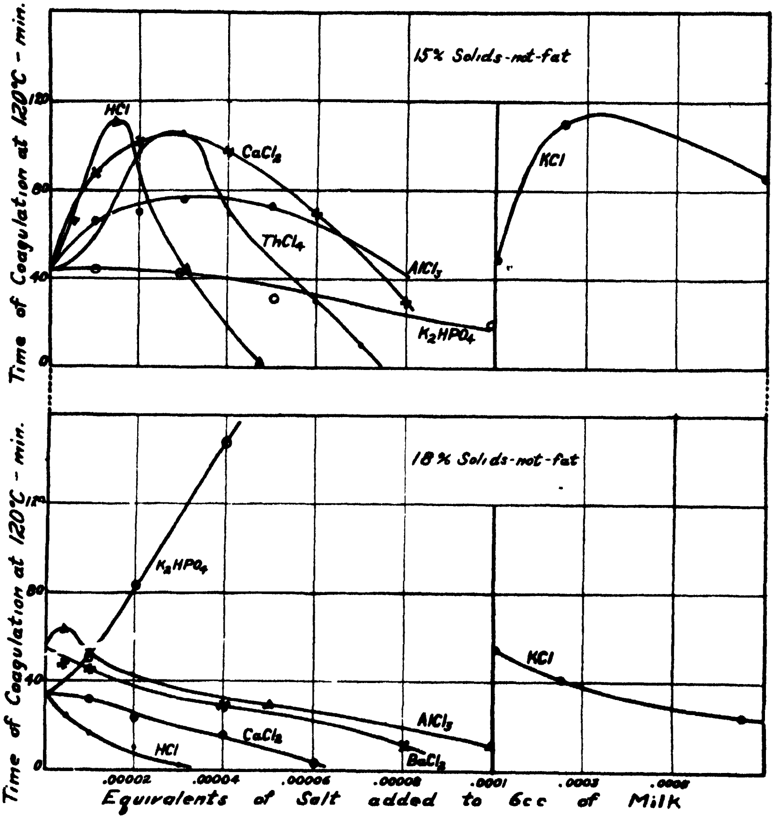

FIG. 15. THE EFFECT OF THE ADDITION OF VARYING AMOUNTS OF DIFFERENT ELECTROLYTES UPON THE HEAT STABILITY OF TWO DIFFERENT TYPES OF EVAPORATED MILK. ALL MILKS WERE FOREWARMED TO 95° C.

It has already been shown that the critical concentration range is 10.5 to 14 per cent solids, which is below that of each of these milks.

Figure 16 shows clearly the effect which different ions may exert upon the heat stability of an evaporated milk. The opposing effect of the calcium and phosphate ions upon stability as first shown by Sommer and Hart (7) is demonstrated and extends almost over the entire range of salt additions practically possible for each milk. It is interesting to note that where the added ion increases stability, this additional heat resistance is very great, amounting in some cases to two or three times the original coagulation period of the milk.

Cations other than calcium can have the same effect as this ion, a fact which clearly demonstrates that the action of added calcium ions in changing the heat stability of a milk is not the result of the calcium itself but rather of the positive charges which the ion carries. It would, therefore, appear that in place of using Sommer and Hart's term of salt-balance, the necessary condition which must exist to secure maximum stability in a milk could better be described as a balance in particle charge. The effect of strongly positive cations is opposed to the action of heavy anions such as citrate or phosphate. Evidently there is a critical balance in the charge on the particle which is the criterion of maximum stability.

The work of Sommer and Hart (2) has shown that many unstable milks can be increased in stability by additions of calcium or of phosphate ions, respectively. It was believed by these workers that if a fresh milk was stabilized by either one of the above mentioned ions, the evaporated product of such a milk would also be stabilized in a similar manner. While this line of reasoning may apply to some milks, it is not typical in all cases as shown by the previous as well as the following experiments.

The effect of additions of $CaCl_2$ and of K_2HPO_4 upon the heat stability of the fresh and concentrated milks of four individual cows and of two composite herd milks are tabulated in table 1. The reaction of the fresh milks toward alcohol is also given.

In every case the addition of a calcium salt decreased the stability of these milks when they were of 7.5 per cent solids-not-fat concentration, while the addition of a phosphate had the opposite effect. This has been found to be generally true with very few exceptions.

However, when the milks were condensed to give a solids-not-fat concentration of 18 per cent, the addition of the calcium salt decreased the stability of the milks from Cows 271, 812 and 807, while the milk from Cow 280 and the herd milks were increased in stability. Addition of the phosphate increased the stability of the milks from Cows 812 and 807, and decreased that of the mixed herd milks.

Complete data upon the action of different electrolytes when added to unevaporated and evaporated milks of these types are plotted in figure 16.

The curves for the milks of each cow were all obtained from the same lot of milk from that cow. The concentrated milks were forewarmed to 95° C. for 10 minutes before evaporation. The directly opposite manner in which the fresh and concentrated milks from both cows react toward the added salts is very striking.

The fresh milk of Cow 280, which was alcohol negative, was increased in stability by the addition of K_2HPO_4, while both HCl and $CaCl_2$ rendered

TABLE 1

Effect of $CaCl_2$ and K_2HPO_4 upon the time of coagulation of fresh and concentrated skimmed milks from the milks of different cows. Concentrated milks were forewarmed to 95° C. before evaporation. Solids-not-fat percentages are those obtained after dilution with water or salt solution

QUANTITY OF SALT SOLUTION ADDED TO 6 CC. OF MILK	COW 271 ALCOHOL +		COW 812 ALCOHOL —		COW 807 ALCOHOL +		COW 280 ALCOHOL —		NO. 1 MIXED HERD MILK		NO. 2 MIXED HERD MILK	
	7.5% S.N.F.	15% S.N.F.	7.5% S.N.F.	15% S.N.F.	7.5% S.N.F.	18% S.N.F.	7.5% S.N.F.	18% S.N.F.	7.5% S.N.F.	15% S.N.F.	7.5% S.N.F.	15% S.N.F.
Equivalents	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
No salt	105	14	301	214	127	34	218	23	212	44	243	41
$CaCl_2$												
.00001	91	11	263			32		31		88		
.00002	78	9	251	197	115	23	196	44	174	102	209	75
.00004	60	6	220	174	60	16	171	119	144	98	176	89
.00006	38	<5	211	144	0	<5		92		70		58
.00008	<5		203					66	118	30		
.0001									5			
K_2HPO_4												
.00001		14		214		50		12		46		
.00002	113	15		219	132	83		12		45	305	30
.00004	118	17		224	138		310	15	285	36		
.00006	127	18	385	230		230		17			339	27
.0001						245		18	350	22		

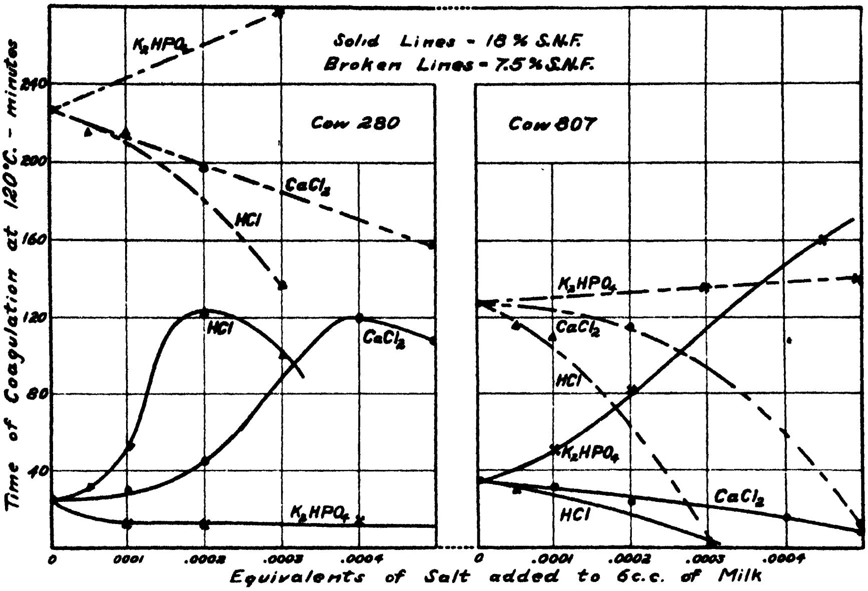

FIG. 16. THE EFFECT OF THE ADDITION OF VARYING AMOUNTS OF DIFFERENT ELECTROLYTES UPON THE HEAT STABILITY OF NORMAL AND EVAPORATED SAMPLES OF TWO DIFFERENT TYPES OF MILK.

it less stable. After this milk was concentrated the effect of these same ions was reversed, a very great increase in stability was produced by additions of $CaCl_2$ and HCl and a decrease by additions of K_2HPO_4.

The milk of Cow 807, which was alcohol positive, behaved in an exactly opposite manner to the milk of Cow 280, as is shown in figure 16.

Sommer and Binny (8) found that by increasing the magnesium or calcium content of a milk the alcohol test became positive, while additions of KCl, NaCl, citrates or phosphates did not cause a positive alcohol reaction. It seemed, therefore, possible that the two different types of milk used to obtain the data in figure 15 might be identified by their alcohol reactions. Accordingly, the milks of some thirty cows which were available for experimental purposes were tested to determine their reaction to alcohol. Unfortunately, Cow 807 was the only animal in this group that was producing alcohol positive milk. It was necessary, therefore, to postpone further work along this line until such a time as more alcohol positive milk might become available. However, the data on the milks of Cows 812 and 280, shown in table 2, both of which were alcohol negative, show that they reacted in different manners when calcium ions were added.

The data presented show quite conclusively that the stability of a milk to various ions is not always reflected in that of its evaporated product. They indicate also the probable reason for the possible use of phosphates in different types of milk without disastrous results. In those cases wherein the addition of phosphate does not stabilize the evaporated product, the stability is not materially decreased. Consequently, the general use of a phosphate stabilizer might at one time be very beneficial while at other times it might produce no increase in stability, nor would it prove decidedly harmful.

With respect to the qualitative, as well as the quantitative, effect of various ions upon the stability of milks the data indicate that comparisons should be made only when comparable quantities of electrolytes, from the standpoint of their effectiveness, are used. For example, results obtained with the use of quantities of an electrolyte in a range wherein they are extremely effective cannot be compared with results obtained with quantities of an electrolyte that lie within the range of little effectiveness. Results obtained under such conditions are apt to lead to erroneous interpretations of the relative value of various electrolytes as stabilizing or destabilizing agents. For accurate interpretation of results it is, therefore, necessary to obtain data over a wide range of concentration of the electrolyte under consideration.

SUMMARY

The variations in the time of coagulation with variations in the temperatures used have been shown to be of a logarithmic nature. However, these variations in the time of coagulation are less with milk of 9 per cent solids-not-fat than they are when milk of 18 per cent solids-not-fat is used. The results indicate also that the use of temperatures of from 110° to 120° C. yield results of greatest practical value. Higher temperatures are apt to give results wherein the errors are large.

2. The data presented upon forewarming also indicate that the concentration of solids-not-fat is an important factor in varying the relative effects of this procedure. The results show that forewarming at high temperatures lowers the stability of those of higher solids-not-fat concentration. Hence, in any comparison of results obtained with milks of different solids-not-fat concentration similar treatment with respect to forewarming must be assured.

These results indicate also that the relationships established by forewarming are not altered by the addition of electrolytes except in degree of stability. It appears, therefore, that forewarming does not affect stability merely through changes in the equilibria of the soluble salts, as the stability cannot be restored by the addition of electrolytes in those cases wherein it has been reduced.

3. The results show conclusively that the heat stability of a milk of 18 per cent solids-not-fat concentration cannot be predicted from the heat sta-

bility of the original milk. They show further why the use of phosphates as a "neutralizer" has not led to serious trouble. In those cases wherein its use has not materially improved stability its use has not greatly reduced it.

4. The existence of two types of milk, one stabilized by the addition of positively charged electrolytes and the other stabilized by the addition of negatively charged electrolytes, has been confirmed. The effect of the addition of various electrolytes to these types of milk has been studied over a wide range of concentration and it has been shown that in the lower concentrations they are most sensitive to the effect of di- tri- and quadrivalent ions and the monovalent H-ion while in the higher concentrations the monovalent K-ion is also very effective. The relationships of these effects are similar to those found for As_2S_3 sols. Hence it again seems to have been shown that the calcium caseinate suspension in milk is analogous to hydrophobic sols in its reactions with electrolytes at higher temperatures. Increased stability has been shown to result from the addition of citrates and phosphates to a milk stabilized by negatively charged electrolytes. Finally, complete data have been presented upon the effect of various electrolytes upon the two types of milk at 9 and 18 per cent solids-not-fat concentrations.

In general, a study of the data presented shows clearly that in studies on coagulation careful consideration must be given to phase concentrations and that conclusions drawn from work on milk of one concentration are not applicable to other milks of widely different concentrations. Furthermore, at the lower concentrations of solids-not-fat various electrolytes exhibit critical concentrations or ranges wherein their effect is great. Hence, any work which does not include results over a range of concentration of electrolyte as well as range of concentration of the sol used is apt to lead to erroneous conclusions.

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THE INCORPORATION OF AIR DURING THE HOMOGENIZATION OF CREAM AND OF ICE CREAM MIXES

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It is not generally known that during the homogenization of cream and ice cream mixes there may be an increase of volume (exclusive of surface foam) due to the incorporation of a small amount of air. This air incorporation is not necessarily permanent but may persist through the cooling process and until after the fluid is stored. Apparently the phenomenon is met with but occasionally and causes no difficulty except as it may cause considerable inconvenience in obtaining an accurate inventory of the stored cream. It is the belief of manufacturers that the condition is met with when defective packings of valves in the homogenizer permit air to be drawn into the machine and mixed with the fluid while processing. In an ice cream factory a number of years ago this volume increase of cream was estimated to be from four to five per cent of the original volume. The volume increase has been variously estimated to amount to as much as 15 per cent.

There seems to be no written record of the amounts of air that can be incorporated in cream or ice cream mixes during processing nor has any consideration been given to possible effects upon air incorporation of such factors as the temperature and pressure of homogenization. For that reason a series of experiments was undertaken to determine in a general way the conditions favorable or unfavorable to the incorporation of air in a fluid during homogenization and the amount of volume increase resulting under the conditions of the tests.

EXPERIMENTAL

The purpose of the tests was to determine the amount of air incorporated in creams and ice cream mixes during the homogenization process as compared with unprocessed control samples.[1] The factors considered for cream were homogenization pressure, homogenization temperature, the clogging of the homogenizer valves, and fat content. In the case of ice cream mixes the factors considered were fat content, homogenization temperature and pressure.

The amount of air incorporated is indicated by the increase in volume of the liquid under test. This volume is most easily measured by noting

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[1] A 90-gallon Progress Homogenizer with gravity feed through standard fittings was used in this experiment. The valves in this homogenizer are in good condition.

the change of density of the fluid during the course of the experiment as compared with its original density. The usual procedure was to homogenize the fluid, cool it on a surface tubular cooler, or if too viscous to flow readily, to cool it in a tank of ice water. Samples were then taken in weighed graduated 500 ml. flasks, brought to a uniform temperature, and the liquid level was adjusted to the mark and the flasks re-weighed.

The presence of foam made it very difficult to fill the flasks by pouring the cream into them, as foam would pour with the liquid cream. This difficulty was overcome by drawing the cream into the measuring flask from the bottom of the container.

The per cent change in volume of the liquid under experiment was calculated from the equation:

$$\text{Per cent change in volume} = \frac{\text{Density of control}}{\text{Density of treated material}} - 100$$

An attempt was made to use a Westphal balance in determining the density but the high viscosity produced in some of the creams made it impossible to obtain accurate measurements by this method.

EXPERIMENTS

Three creams containing 20, 25, and 30 per cent fat, respectively, were each pasteurized at 145° F. for 30 minutes; then each was divided into five separate lots. The first lot (Control) was cooled without homogenization; the second lot was homogenized at 145° C. with 1000 pounds pressure; the third lot was homogenized at 145° F. with 3000 pounds pressure; the fourth lot was cooled to 128° F. and homogenized with 3000 pounds pressure; and the fifth lot was cooled to 110° F. and homogenized with 3000 pounds pressure.

The volume increases are given in table I.

These data show that neither changes in pressure nor small changes in temperature of homogenization are important factors in affecting the volume or density of homogenized cream containing 20 to 30 per cent fat. It is evident, however, that if extremely low temperatures are used the volume of the cream may be appreciably increased. There is a greater tendency for air incorporation in the higher fat creams but this tendency is not particularly marked and is apparently not always met with as evidenced by the next series of experiments.

The effect of faulty flow of cream into the homogenizer on air incorporation was investigated by closing one of the intake valves. In this way only two valves of the homogenizer were pumping cream normally while the other might possibly be pumping air into the cream. This caused the gauge hand of the homogenizer to be unsteady and jump from 1000 to 2500

TABLE I

Per cent volume increase of creams containing 20, 25 and 30 per cent fat

PER CENT FAT	LOT NO.	HOMOGENIZATION		EXPERIMENTS			
		Pressure	Temperature	No. 1	No. 2	No. 3	Average increase
				Volume increase	Volume increase	Volume increase	
		Pounds	°*F.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
20	1		145				
	2	1000	145	.00	.06	.04	.03
	3	3000	145	.38	.24	.18	.26
	4	3000	128	.46	.45	1.04	.66
	5	3000	110	.98	.69	3.08	1.59
25	1		145				
	2	1000	145	.02	.04	.00	.02
	3	3000	145	1.00	.04	.08	.38
	4	3000	128	.09	.80	1.24	.72
	5	3000	110	1.98	1.86	2.98	2.28
30	1		145				
	2	1000	145	.24	.26	1.32	.61
	3	3000	145	1.39	.32	1.52	1.08
	4	3000	128	1.92	.20	.88	1.00
	5	3000	110	4.25	1.50	1.40	2.38

pounds with the uneven action of the three valves. The data are recorded in table II.

TABLE II

Effect of closing one homogenizer intake valve on air incorporation in cream

Per cent fat in cream	VOLUME INCREASE OF CREAM OF DIFFERENT PER CENT FAT CONCENTRATION		
	20	25	30
	Per cent	*Per cent*	*Per cent*
Homogenized 145°F. (2500 lbs.)	3.15	2.66	1.28
Homogenized 110°F. (2500 lbs.)	11.16	5.18	3.44

More air was incorporated in these creams than in those of the preceding series of tests when the homogenizer valve was not blocked. However the amount decreased as fat increased. It is evident therefore that defects in the homogenizer action may give abnormal air inflations in cream.

No report has ever reached the authors of difficulties in manufacture due to air incorporation in ice cream mixes during homogenization. However, in order to determine whether or not homogenization did tend to incorporate air in ice cream mixes tests were made on two different types of mixes, *i.e.*, high and low fat content mixes. The data is given in table III.

TABLE III

Per cent air incorporated in homogenized ice cream mixes of 16 and 12 per cent fat content

LOT NO.	HOMOGENIZATION		ICE CREAM MIX	
	Pressure	Temperature	16 per cent fat	12 per cent fat
	Pounds	*°F.*	*Per cent*	*Per cent*
1	Control		0.00	0.00
2	1000	145	.26	.34
3	3000	145	.26	.14
4	3000	128	.88	.04
5	3000	110	1.06	.06

Here the air incorporation is not markedly different in amount between the ice creams of high and medium fat content except in cases where the temperature was lowered in homogenization of the 16 per cent mix. The lower temperatures, however, did not seem to affect the inflation of the lower fat mix.

DISCUSSION AND SUMMARY

In this series of experiments conducted to determine the amount of air that might be incorporated in creams and ice cream mixes during homogenization the maximum increase in volume that was attained under normal conditions was 4.25 per cent. When one homogenizer valve was clogged the maximum increase in volume amounted to 11.16 per cent. These values were not attained under normal conditions unless the homogenization temperature was unusually low. At the usual homogenization temperatures, or at those near pasteurization temperature, no significant increases in volume were obtained in either the cream or ice cream mixes.

SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. I. THE CHURNABILITY OF ARTIFICIAL EMULSIONS PREPARED WITH THE KNOWN EMULSIFYING AGENTS OF CREAM*

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The nature of the material adsorbed on the surface of the fat globules in cow's milk has been the subject of a number of investigations. An excellent review of the literature to 1925 is given by Hattori (1). With one exception (2), all the researches have been concerned with the identification of an adsorbed protein. Since the latter compounds are known to be good emulsifiers, it is not surprising that each of the major proteins found in cow's milk should, at one time or another, have been assumed to constitute the active fat stabilizer. Different methods of isolation, however, have yielded products varying in composition and properties. The results published, therefore, have not been uniform. The active fat stabilizer has been reported to be a glycoprotein (3), casein (4), albumin (5), a mixture of a globulin-like protein and phospholipides (2), and a new protein (1) differing in composition from any of the other known milk proteins.

In order to ascertain whether some of these materials might constitute the sole fat stabilizer in cream, artificial emulsions of butterfat stabilized by various milk constituents were prepared. Since this investigation was concerned only with the fat emulsion of cream and the churnability of cream is the most characteristic reaction of the fat component, each of the artificial emulsions was subjected to the churning test.

In preparing the butterfat emulsions, sufficient melted filtered butterfat to give a four per cent milk was added to a one per cent dispersion of the emulsifier at 100° F. This was passed through a Gaulin homogenizer, at such a pressure as to yield a butterfat dispersion resembling microscopically as nearly as possible that of whole milk. The best emulsions were usually obtained at a pressure of 400–500 pounds. The milk was separated in a McCormick-Deering hand separator, operating at normal speed, the cream standardized with its own skimmilk to 30–35 per cent butterfat, cooled over night at 40° F., churned at 55°–57° F. in a glass Dazey churn

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of suitable size, at a crank speed of 90–120 r.p.m. (320–430 r.p.m. of the paddle), and a microscopic examination of the butter made. Each butter was worked slightly with wooden butter paddles before the microscopic examination was made. The type of emulsion present in the churned product was determined by the microscopic method devised by Palmer (6). When the water droplets in a water-in-oil emulsion are in focus and the objective is lowered, the water droplets show bright centers just before they disappear. With oil-in-water emulsions, the oil globules show bright centers when the objective is raised.

The same general procedure was followed in the preparation, churning and examination of all the emulsions.

Fresh buttermilk, calcium caseinate, serum globulin, lactalbumin and a crude egg yolk phospholipide were used as the emulsifying agents. In every case a very good dispersion of butterfat in water was obtained. The macroscopic and microscopic appearance of the milks resembled very closely that of whole cow's milk and each one was stable after separation. However, there was considerable difference in the ease of separation and churnability of the creams.

BUTTERFAT DISPERSED IN BUTTERMILK

According to the foam theory of churning, (7) the foaming compound in milk and the fat stabilizing substance are identical and remain incorporated in butter as a rigid, solid, continuous phase of dehydrated colloid. The fat emulsifying agent therefore would not appear in the buttermilk. On the other hand, if cream is considered an oil-in-water type of emulsion and butter a water-in-oil type, one must assume an inversion of phases taking place at some stage of the butter making process. The fat stabilizing material, then, must be removed from the globules during the churning process to permit coalescence of the fat to form the continuous phase in butter. Although some of the material adsorbed on the surface of the globules might remain incorporated in the water droplets in the butter, at least part of it should appear in the buttermilk. In view of these contradictory theories of churning, it seemed desirable to determine whether buttermilk contained materials which would stabilize a butterfat emulsion comparable in all respects to the butterfat emulsion of normal cow's milk.

For this purpose, melted filtered butterfat and fresh buttermilk at 100° F. were passed through a Gaulin homogenizer, separated, cooled and churned according to the procedure described above. The milk separated and churned very well. Microscopic examination of the cream showed no clustering of the fat globules. A small amount of foam was produced during the churning process. Butter formed and gathered in a normal way in 27 minutes. There was nothing abnormal about the appearance or behavior of the milk. In view of the results obtained with other emulsions, it appears probable that the buttermilk used as the dispersions medium contained some of the same material which stabilized the fat globules in the original whole milk. However, the material had not been denatured or altered in such a way as to destroy its physical-chemical emulsifying property.

BUTTERFAT DISPERSED IN CALCIUM CASEINATE SOL

The casein used in the preparation of the calcium caseinate milk was grain-curd casein which had been reprecipitated from ammonium hydroxide solution, rinsed, washed with isoelectric distilled water by soaking in new wash water for exactly one week, and then extracted with alcohol and ether. The casein was ground with an excess of calcium hydroxide, the excess alkali removed by precipitation with oxalic acid, and the mixture supercentrifuged. Two per cent phosphoric acid was slowly added to the supercentrifuged material by means of a capillary pipette and mechanical stirrer until pH 6.47 was reached.

Microscopically the butterfat dispersion prepared at a pressure of 300–400 pounds with the calcium caseinate-calcium phosphate stabilizer was very good. However, considerable difficulty was experienced in separating the milk. The emulsion showed no tendency to break but a thick, almost solid, cream collected between the discs of the separator, clogging the openings and preventing any liquid cream from flowing out. By scraping the thick cream from the discs and bowl of the separator and mixing it in a mortar with its own skimmilk, a stable uniform cream was again readily prepared.

During the churning process a coarse foam which completely filled the churn formed at once. The foam became finer as the crank speed of the churn was increased from 90 to 120 r.p.m. Butter granules formed but did not gather even after several hours of churning. Microscopic examination of the foam showed partially churned material, that is, material in which very large fat globules and irregular masses of fat containing minute water droplets, and a regular dispersion of fat-in-water were visible.

Various amounts of lactic acid were then added to fresh portions of the cream and the time and ease of churning noted.

The addition of lactic acid reduced the time of churning and aided in the gathering of the butter granules. The fact that most of the fat globules in the second cream were clustered did not reduce the churning time below that of the fourth cream in which there were no clusters present. Although the butter granules gathered in the case of all the acid creams, they did so very slowly and a great deal of fat was lost in the buttermilk, *i.e.*, 6–7 per cent. Increasing the acidity to pH 4.6 theoretically should decrease the stability of the butterfat emulsion, for the surface activity of the adsorbed casein would be at a minimum at the isoelectric point. In general, this was found to be true. The time of churning, taken as a measure of the stability of the emulsion, decreased with an increase of acidity.

The stability of the emulsion, although lessened, was not decreased to the breaking point during the increase in acidity which passed through the isoelectric point. There was, no doubt, a reversal of the charge on the surface of the globules between pH 5.2 and 2.2. The cream at pH 2.2 foamed only slightly. The production of an excessive amount of foam which decreased markedly before butter granules formed was characteristic of all the other calcium caseinate creams. This appears to be in contradiction to Rahn's theory that foaming is essential for churning. The cream yielding the smallest amount of foam churned the quickest. None of the churnings, however, resembled those of normal cream. The results of the churning experiments are given in table 1.

TABLE 1

Churnability of calcium caseinate cream

FAT CONTENT OF CREAM	CHURNING TEMPERATURE	CHURNING TIME	pH OF BUTTERMILK	FAT IN BUTTERMILK
Per cent	°*F.*			*Per cent*
22.5	52.8	2 hrs. 30 min.*		
22.7	57.2	2 hrs. 10 min.	6.0	6.0
32.0	57.2	1 hr. 20 min.	5.2	
27.0	56.3	35 min.	2.2	7.0

* Butter granules did not gather.

BUTTERFAT DISPERSED IN LACTALBUMIN SOL

The lactalbumin solution was prepared from a sample of soluble lactalbumin powder[1] obtained from the Research Laboratories, Bureau of Dairying, Washington, D. C. The milk prepared by dispersing butterfat in the lacalbumin solution separated much more easily than the calcium caseinate dispersion, but not as well as the fresh buttermilk dispersion. After standardization, no clusters were visible microscopically. Churnings were also made of the cream to which varying amounts of lactic acid were added. During churning a large volume of coarse foam appeared at first. This gradually became finer, decreasing in volume and becoming a uniform whip before butter granules formed. The churned products showed typical butter structures, although there was considerable difference in the degree of dispersion of the water droplets. All the products were very light in color, the most acid cream yielding a perfectly colorless product. The latter contained extremely small water droplets. This observation for an artificial cream is in accord with the well known fact that the color of butter is, in part, dependent on the distribution of the water in the butter. The churning results are given in table 2.

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[1] The lactalbumin powder contained 35.37% protein (N × 6.38), 0.195% ash and 64.44% lactose (by difference). We wish to acknowledge the kindness of Dr. L. A. Rogers, who furnished us with this product.

TABLE 2

Churnability of lactalbumin cream

FAT CONTENT OF CREAM	CHURNING TEMPERATURE	CHURNING TIME	PH OF BUTTERMILK	FAT CONTENT OF BUTTERMILK
Per cent	°*F.*		*Per cent*	*Per cent*
36.5	57.2	32 min.	6.66	4.3
36.5	57.2	33 min.	4.85	3.4
27.5	57.2	35 min.	3.24	2.5

BUTTERFAT DISPERSED IN GLOBULIN SOL

Since lactoglobulin and serum globulin have been shown to be identical (8) (9), a globulin dispersion was prepared by dispersing commercial boval[2] in water containing a small amount of ammonium sulphate. After supercentrifuging, the globulin was precipitated with half saturated $(NH_4)_2SO_4$, again supercentrifuged, redispersed in water and reprecipitated. The globulin dispersion was then dialyzed in cellophane bags against running tap water until almost free from sulphates.

The globulin milk separated better than the calcium caseinate milk but not well enough to be considered normal. It yielded a heavy cream, some of which collected between the discs of the separator. It remained stable, however, and mixed readily with the thinner portion and was then standardized to 35 per cent butterfat. During the churning process, a coarse foam formed at the beginning quite similar to that produced by the casein creams. One cream, which required an hour and 43 minutes to churn, behaved more like a casein cream than any of the others. Butter granules formed on the sides of the churn long before churning was complete. The butter structure was poor and the loss of fat in the buttermilk was about 15 per cent. All the other churned products had a typical butter structure but were extremely pale in color due to the presence of very fine water droplets. The difference in the behavior of the cream at pH 5.27 cannot be explained. Acidification had little effect on the churning time of the other creams. The churning experiments gave the results shown in table 3.

BUTTERFAT DISPERSED IN PHOSPHOLIPIDE SOL

The phospholipide content of cow's milk has been investigated by a number of workers, the amount reported varying from .040 to .1173 per cent. According to Glikin (10) the major part of it passes into the cream but when the cream is churned, only a trace is retained in the butter (11) (12). Most of it appears in the buttermilk. Palmer and Samuel-

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[2] Dried blood serum.

TABLE 3

Churnability of globulin cream

CHURNING TEMPERATURE	CHURNING TIME	PH OF BUTTERMILK	FAT CONTENT OF BUTTERMILK
°F.			*Per cent*
56	27 min.	6.84	5.0
57	1 hr. 43 min.	5.27	15.0
57	30 min.	4.19	4.4
57	30 min.	3.40	5.0

son (2) also found phospholipide-like substances in the material isolated by them from the fat globules in washed cream. It appears likely, therefore, that a part of the phospholipide in milk is closely associated with the fat globules. With this in mind, a butterfat dispersion stabilized by phospholipide was prepared.

Since the amount of phospholipide in cow's milk is so small isolation from this source was impractical. A more feasible procedure was to secure the phospholipide from a product rich in lecithin. While egg yolk lecithin might differ from the mixed milk phospholipides in chemical composition insofar as the fatty acids are concerned, it still possesses the property of an excellent emulsifying agent. Crude phospholipide was prepared from fresh egg yolks. The water, fat, sterols and other acetone soluble substances were removed from the yolks by repeated extraction with acetone until the extract was practically colorless. The moisture-free, fat-free, sterol-free residue was extracted with hot 95 per cent ethyl alcohol and the solvent distilled off under reduced pressure. The crude phospholipide was recovered from the residue. A one per cent aqueous dispersion served as the emulsifying agent.

The phospholipide milk separated more nearly like natural cow's milk than any of the other butterfat dispersions prepared. The cream, however, did not give normal churnings. Butter granules formed but did not gather well, about ten minutes being required for the latter process. The butter did not "break." Microscopically, two of the products showed peculiar structures with very small water droplets dispersed in fat together with many fat globules still intact, closely packed and massed together. The best product microscopically was obtained at pH 4.75.

It was apparent in two of the products that the inversion of the oil-in-water type of emulsion was not complete. The phospolipide appeared very effective as an oil-in-water stabilizer. According to Feinschmidt (13), Fugii (14), Price and Lewis (15) and Remesow (16), the isoelectric range for lecithin lies between pH 2.2–6.3. Although the churnings were made in this range where lecithin was exhibiting its minimum surface activity, the emulsions were not completely inverted. The results of the churning experiments are given in table 4.

TABLE 4

*Churnability of phospholipide cream**

CHURNING TEMPERATURE	CHURNING TIME	pH OF BUTTERMILK	FAT CONTENT OF BUTTERMILK
°*F.*			*Per cent.*
54	30 min.	6.69	2.9
56	25 min.	4.75	1.9
57	60 min.	3.10	About 12.0

* Fat content of cream was 31 per cent.

BUTTERFAT DISPERSED IN GLOBULIN AND PHOSPHOLIPIDE SOL

In the preceding experiments, only single substances, calcium caseinate, lactalbumin, serum globulin, or egg yolk phospholipide were used as the fat stabilizer. It seemed possible that combinations of these would yield emulsions which behave more like normal cow's milk both on separation of the cream and churnability of the cream than those stabilized by one constituent. One combination only was tried. Palmer and Samuelsson (2) concluded that protein and phospholipide existed in a ratio of approximately 1: 6 in the "membrane" material isolated by them. The skimmilks from the globulin and phospolipide creams were therefore mixed in the ratio of 1: 6 and melted filtered butterfat dispersed in the mixture.

The milk separated well but again the acid cream failed to yield typical churned products. The microscopic structure of the products was slightly better than those of the phospholipide cream. The results of the churning experiments are given in table 5.

"WASHED" CREAM

Storch (3) was the first to observe that cream would churn normally when obtained by diluting milk with water and passing it through a centrifugal cream separator. He observed that the stability of the cream was not lessened by repeating the washing 4 times using 4 volumes of water to 1 of cream at each washing. Palmer and Samuelsson (2) verified these observations, starting with fresh cream instead of whole milk.

We have again confirmed Storch's findings, using even more drastic manipulation. We have repeatedly prepared a stable cream which has been freed from milk plasma by dilution and reseparation. Four volumes of distilled water, temperature about 100°–110° F.,[3] to one volume of cream were employed at each dilution and the number of such dilutions repeated as many as 8 times without destroying the cream emulsion. Sepa-

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[3] A wash-water temperature of about 105°F. seemed to give the best results.

TABLE 5.

*Churnability of globulin-phospholipide creams**

CHURNING TEMPERATURE	CHURNING TIME	PH OF BUTTER MILK	FAT CONTENT OF BUTTERMILK	GENERAL AND MICROSCOPIC APPEARANCE OF PRODUCT
°*F.*			*Per cent*	
57	5 min.	6.6	3.6	Yellow in color. Fat continuous phase. Wide distribution in size of water droplets.
57	17 min.	4.2	3.7	Light yellow in color. Fair distribution of water-in-fat. All water not in droplet form.
56	20 min.	3.9	5.6	Light yellow in color. Wide distribution of water droplets. All water not in droplet form.
57	47 min.	3.4	8.0	Light yellow in color. Fair distribution of water droplets. All water not in droplet form.

* Fat content of cream was 35 per cent.

ration of the cream was effected in each instance in the present studies by means of a Sharples supercentrifuge geared down to operate at a speed of about 10,000 r.p.m. A volume of wash water from the 4th to 8th washings equal to one and one-half times the volume of original cream, gave either a negative or faint biuret test on evaporation to dryness.

The churnability of the creams prepared in this manner was tested in each case in the following manner. The washed cream was standardized to a fat content of 35 per cent using distilled water, the cream from the final washing usually exceeding this fat percentage. The standardized cream was tempered over night at a temperature of 38°–40° F. and churned the following morning at temperatures ranging from 44°–55° F., depending on the season. The churn employed was either a 1 or 2 quart glass Dazey churn or a small metal barrel churn when larger volumes of washed cream were obtained.

Inasmuch as these churnings were carried out for another purpose, the results of which will be reported in a later paper, the data on these churnings are not as complete as might be desired. The available data are summarized in table 6. In addition certain interesting observations may be mentioned.

Although the cream prepared by washing is a stable emulsion having both the macroscopic and microscopic appearance of normal cream, its stability is somewhat less than normal unwashed cream in that a small amount of churning takes place in the passage of the diluted cream through the centrifuge, particularly after the 4th washing. This stability is further decreased by increasing the acidity, as seen in experiment 2, table 6. This cream showed a much greater tendency to churn during its washing and butter began to form at once in the churn.

TABLE 6

Churning experiments with washed cream

EXP. NO.	VOLUME ORIGINAL CREAM	CONDITION OF ORIGINAL CREAM	NUMBER OF WASHINGS	FAT CONTENT WASHED CREAM	FAT CONTENT CHURNED CREAM	TIME REQUIRED FOR CHURNING	COMPOSITION BUTTER MILK
	liters			*per cent*	*per cent*	*minutes*	
1	1.0	fresh-sweet	8	49.0	35	20	0.36% T.S.
2	1.0	0.45% acidity	6		35	at once	1.77% T.S.
3	3.8	fresh-sweet	6		35	30	
4	8.0	fresh-sweet	6		35	slowly	
5	5.7	fresh-sweet	6	31.0	31	readily	
6	15.4	fresh-sweet	6	56.0	35	readily	
7	15.4	fresh-sweet	6	49.5	35	readily	

Special attention was given to the microscopic structure of the butter obtained in experiment 4, in which the butter formation was not as rapid as in the previous experiments. The structure was, however, entirely normal, showing the microscopic water drops well dispersed in the continuous fat phase.

The buttermilk from the churning in experiment 6 was studied microscopically both before and after passing it through the Sharples supercentrifuge at high speed (30,000 r.p.m.). Before centrifuging considerable fat still in globular form was observed, as well as some butter particles, showing that the churning had not been very exhaustive. This was true for most of these experiments but cannot be employed as criticism because they were not planned from this point of view. After supercentrifuging the buttermilk, practically no fat could be observed microscopically. In experiment 7, however, the churning must have been quite exhaustive because practically no fat was thrown out in the bowl when the buttermilk was supercentrifuged at high speed.

SUMMARY

Fresh buttermilk contains a substance or substances having the ability to stabilize oil-in-water emulsions. It is probable that a portion of this material comes from the surface of the fat globules in cream during the churning process. The butterfat-in-buttermilk dispersion resembles whole milk in every respect, in general appearance, microscopic structure, cream separation and churnability of the cream.

Stable emulsions of butterfat can be prepared in dispersions of calcium caseinate, lactalbumin, globulin and phospholipide. These resemble whole cow's milk in general appearance, formation of a cream layer and microscopic structure. Each shows an abnormal behavior either on separation of the cream or in the churability of the cream. The best separation is obtained with a phospholipide stabilized emulsion and the best churning with a lactalbumin stabilized emulsion.

Since cream freed from milk plasma churns normally, it seems justifiable to draw conclusions from the emulsions which likewise lacked most of the constituents of normal milk plasma. It is apparent from the preceding churning experiments that no one of the milk constituents used produces emulsions which behave entirely like normal cow's milk. It, therefore, does not appear probable that any one of the substances tested constitutes the sole material adsorbed on the surface of the fat globules in normal cow's milk.

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METHODS FOR USE IN THE BACTERIOLOGICAL EXAMINATION OF DRY MILK AND RELATED POWDERS

FOREWORD

The American Dairy Science Association, through its committee on bacteriological methods, is formulating bacteriological procedures useful in controlling the quality of dairy products. This committee is acting through sub-committees appointed from men in the organization who have had experience with the bacteriological analysis of various dairy products. Obviously, the formulation of such methods should not be left to the arbitrary decision of a committee, but should be the result of suggestions and criticisms coming from all interested parties, whether or not they are members of the American Dairy Science Association. The committee, therefore, wishes to serve as a center about which methods satisfactory to the largest number may be evolved.

The purpose of this preliminary report is to submit an outline of methods for making bacteriological examinations of dry milk and certain related powdered baby foods. These methods will be revised later in accordance with the suggestions received before they are finally adopted by the American Dairy Science Association and included in a general report on bacteriological methods of analyzing dairy products.

METHOD OF SAMPLING

It is very important that all utensils and containers used for sampling milk powder be clean, dry and sterile, and that the work be done as speedily as possible. Milk powder absorbs moisture from the atmosphere very rapidly. Samples should always be taken at points beneath the exposed surface.

If samples are to be taken from a bulk or mass of powder not contained in standard packages it is convenient, with the aid of a sterile tablespoon or wide spatula, to obtain samples of substantially the same size from various points throughout the powder mass. Such samples are to be combined in a suitable sterile container from which a properly mixed composite sample is obtained later.

Sampling of milk powder from standard small package units may be obtained simply by withdrawing the desired amount from the powder avoiding that immediately adjacent to the exterior surfaces. A sterile tablespoon or sterile spatula is very convenient for this purpose.

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If samples are to be obtained from the standard bulk packages such as barrels, drums or boxes containing 25 pounds or more it is desirable to remove the layer of powder adjacent to the barrel head or opening to the depth of about three inches and insert in such a cleared area a sampling tube or device similar to an elongated butter or cheese trier. Such a device may not remove an unbroken column of milk powder but in general a representative sample obtained through the entire depth of the container can be secured by such a device, the efficiency of which is determined largely by its design and the manipulations involved in its use. At least three such "plugs" or columns of powder should be withdrawn from a single container. The number of samples thus obtained from a single lot or consignment will obviously be determined by the requirements to be met.

When it is necessary to prepare a composite sample from the combined portions obtained as above in order that the final sample may be representative of a given quantity of powder, several good sized samples obtained from the various batches should be collected in a container of suitable size in order to permit thorough mixing of the desiccated product in order that the sample to be withdrawn for analysis may be representative of the combined portions. Single or double friction top cans, previously cleaned, and sterilized are recommended for collecting, holding or shipping samples. Variations which may occur in a given quantity of powder can be more readily detected by examining separate samples rather than a composite sample, and, in some cases, this practice is preferable.

PREPARING THE SAMPLE FOR ANALYSIS

If the sample of powder should completely fill the container in which it is collected or received, the entire contents should be transferred to another clean, dry, sterile container of ample size to permit proper mixing. The detailed manipulations for mixing the sample may be left to the discretion of the individual, it being understood that the necessity of uniform mixture is as desirable in this case as in any other wherein a representative sample is desired. It is important during the mixing and handling of the product to prevent extraneous contamination as from a dusty atmosphere, improperly cleaned or unsterilized utensils. The same general precautions as are applied to the preparation of any sample for bacteriological analysis apply also to dry milk.

SUGGESTED METHODS OF ANALYSIS

(*a*) STANDARD AGAR PLATE METHOD.

The standard agar plate method of the American Public Health Association as used for fluid milk may be used for determining the number of organisms in dry milk.

Preparing Dilutions:

It is believed desirable to follow as closely as practical, methods already recognized as standard procedures. For this reason the method of bringing the volume or weight up to 100, as used in the Standard Methods of Milk Analysis of the American Public Health Association, is preferred to the method of adding the material to be tested to 100 cc. or grams as the case may be.

For the one to ten dilution completely dissolve or mix ten grams of the prepared sample in ninety grams of distilled water. In certain instances it may be more convenient to prepare a one to ten dilution by using forty-five grams of water and five grams of powder. If a one to hundred dilution is desired, this may be prepared from the one to ten dilution or one gram may be used with ninety-nine grams of distilled water. The dilutions may be conveniently prepared by weighing the dry sample directly into a bottle containing the dilution water; or into the empty sterile dilution bottle, the required amount of sterile water being added just prior to dissolving the powder for plating. If the latter procedure is followed glass stoppered bottles will be necessary in order to sterilize the bottles by hot air. In the examination of milk powders or compounded infant foods which do not readily form a complete solution with water, it is desirable to facilitate such solution by bringing the temperature of the dilution water to 110–120° F. before mixing with the sample. The plating should be completed within 15 minutes after addition of the water in order to prevent growth in the dilution bottle which would affect the final results.

If lactic acid milk powder preparations are being examined a dilution may be made by using N/10 LiOH as the diluent in order to aid in dissolving the powder. This same type of diluent may be also used for dilutions for the bacteriological examination of powders containing calcium caseinate, although in this case a one to hundred dilution in distilled water is preferable in order to prevent excessive foaming.

In the case of incomplete solution of the milk powders or certain constituents of compounded preparations, the diluted product should be kept thoroughly agitated during withdrawal of the sample with the pipette in order that a uniform sample of the solution and suspended material may be transferred to the petri plate.

Preparing Plates:

Dilutions should be made which will give not more than 300 nor not less than 30 colonies on the plate. The preparation of the media and pouring of the plates should follow the suggestions outlined in the latest edition of the Standard Methods of Milk Analysis of the American Public Health Association (Fifth Edition, 1928).

Incubation Temperature:

In addition to the incubation temperature of 2 days at 37° C. as recommended in the standard procedure, other incubation periods and temperatures may be used for special purposes, such as 5 days at 25° C., 3 days at 30° C., 2 days at 35° C. or 2 days at 55° C. The later temperature is especially useful for estimating the number of thermophilic bacteria which are often present in large numbers due to certain steps in the manufacturing process.

Counting of Plates—Precautions:

Extreme precaution should be exercised in counting the incubated plates in order to differentiate between the bacterial colonies and suspended constituents of the sample transferred to the plates from the initial dilutions. Insoluble milk powders or compounded products containing insoluble material frequently cause residues in the plates which are readily confused with bacterial colonies. Devices for counting the plates with the aid of artificial illumination and low magnification are strongly recommended. Doubtful objects should be examined with higher magnification to determine whether they are colonies.

(*b*) MICROSCOPIC COLONY COUNT (FROST METHOD).

In laboratories already equipped and experienced in counting bacteria in milk by this method, it would seem that this method could be readily used in the routine control of dried milk. Directions as outlined in the Standard Methods of the American Public Health Association should be followed.

The original sample should be diluted as suggested in the methods for the agar plate count. Incubate for 12 to 16 hours at 37° C.

(*c*) DIRECT MICROSCOPIC COUNT (BREED METHOD).

The direct microscopic count applied to milk powders has a certain value for indicating the bacterial character of the fluid milk from which the product was made. Where this method is to be used for purposes of information of this kind it should be carried out according to the directions given in the Standard Methods of Milk Analysis of the American Public Health Association.

The direct microscopic count cannot be relied upon as an adequate procedure for determining the bacterial count of milk powders. It may be of supplementary value in conjunction with certain plating procedures listed herein.

(*d*) EXAMINATION FOR HEMOLYTIC STREPTOCOCCI.

Standard nutrient agar plates to which has been added 0.5 per cent sodium chloride and 2 to 5 per cent defibrinated blood is suggested for use in determining the presence of hemolytic streptococci. The blood should be added aseptically after the agar is melted and cooled prior to pouring the plates. The plates should be incubated for 2 days at 37° C. Examination should be made at the end of 24 to 48 hours.

Suspicious hemolytic colonies should be examined by Gram staining to determine the presence of streptococci. Before definite conclusions can be drawn, reference should be made to standard text books on bacteriology dealing with the differentiation and recognition of hemolytic streptococci.

If conditions permit its use, veal infusion medium will give somewhat better results than the standard nutrient agar for the growth of the hemolytic streptococci. This medium is prepared as follows:

Ground lean veal	500 grams
Distilled water	1,000 cc.

Infuse over night in a cold room. Strain through cheese cloth by pressing. Make up to the original volume. Bring to boiling with frequent stirring and boil until the infusion is clear and the coagulum brown. Strain through cheese cloth and filter through paper. Adjust reaction to pH 6.8 to 7.0. Add 0.5 per cent Difco peptone and 0.5 per cent sodium chloride. The broth should then be autoclaved for 15 minutes at 20 pounds' pressure. This is a higher pressure than that used for the final sterilization and is used in order to avoid precipitates in the finished product. Egg may be added previous to autoclaving if a glass clear medium is desired. Filter and sterilize at 15 pounds' pressure for 15 minutes. Before the final sterilization, 1.5 per cent agar is added and the medium distributed in flasks in readiness for plating. Blood (rabbit, horse, sheep, etc.) may be added aseptically just prior to plating and after the agar has been melted and cooled to 50 degrees C.

(*e*) EXAMINATION FOR THERMOPHILIC BACTERIA.

For the routine determination of the presence of thermophilic organisms plates may be prepared from standard nutrient agar. For investigation or research purposes it is recommended that the following materials be added to each 1,000 cc. of standard agar.

Bacto Trytophane Broth		2.5 grams
Bacto Yeast Extract		1.0 gram
Dextrose		1.0 gram
pH	7.0 ± 0.2	

The plates should be incubated for 48 hours at 55° C. At least 15 cc. of medium should be used in each plate to prevent excessive drying during the incubation. Water should be placed in the incubator in order to reduce the drying of the plates to a minimum.

MEDIA FOR SPECIAL PURPOSES OTHER THAN THOSE LISTED ABOVE

(*a*) Inasmuch as the standard procedure of the American Public Health Association as outlined above (*a*) may not be conducive to the growth of all organisms in milk powder any more than it is to the growth of all organisms in natural fluid milk, the following medium is suggested for special purposes.

Bacto Trytophane Broth	2.5 grams
Bacto Peptone	2.0 grams
Bacto Yeast Extract	1.0 gram
Dextrose	5.0 grams
Agar	15.0 grams
Water	1,000 cc.

Incubation temperature in this case should be for 3 days at 30° C. or at other times and temperatures in accordance with the results desired.

(*b*) Whey agar may also prove satisfactory for obtaining counts required for investigational or interpretative purposes and may be prepared according to methods outlined in Suggested Methods for the Microbiological Analysis of Butter, JOUR. OF DAIRY SCI., Vol. 13, 396–397, 1930.

EXAMINATION FOR YEASTS AND MOLDS

The determination of yeast and mold counts in dried milk, while not a common practice, is used quite extensively in the butter industry. No doubt a more complete study of dried milk from this view-point might give us some valuable information regarding sanitary conditions. (See Suggested Methods for the Microbiological Analysis of Butter, JOUR. OF DAIRY SCI., Vol. 13, 380–405, 1930.)

METHOD OF REPORTING COUNTS

Results should normally be reported per gram of dry powder. If desired they may be transformed to the fluid basis by dividing by the factor used in reconstitution. This is usually 8 parts of water to 1 gram of powder.

In the interest of uniformity of practice and interpretation of data from various sources, the medium used and the time and temperature of incubation should always be reported in direct conjunction with the numerical results obtained on the one gram basis. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation periods and temperatures are used.

CONCLUSION

The views expressed in this report are those of a committee appointed by the American Dairy Science Association. As such they are printed for the general criticism of other members of the American Dairy Science Association and other interested parties.

Separate copies of this report may be secured at cost from the chairman of the Committee on Bacteriological Methods.

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NOTE ON USE OF ALKALINE WATER BLANKS FOR PLATING DRIED MILK PRODUCTS

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In the counting of bacterial colonies in plates prepared from dried milk products considerable difficulty is encountered in distinguishing tiny particles of undissolved protein suspended in the agar from small colonies of bacteria, especially in the powdered acid milk products. Due to this difficulty there are times when erroneous results are reported.

Because of this difficulty, a study was made of methods to eliminate the protein particles without materially affecting the results of the plate count of the product. Since milk proteins are dissolved by alkaline solutions, the effects of different, individual alkalies and various combinations of them were tested for their ability to dissolve the protein particles insoluble in distilled water. More than twenty different alkaline solutions, including the sodium phosphates, were tested upon various kinds of dried milk products. The best results were obtained with lithium hydroxide solutions. Further work showed that M/5 and M/10 LiOH solutions were satisfactory for this purpose. A lack of germicidal action of these LiOH solutions was shown by a comparison of bacterial counts of various powdered milk products obtained by using distilled water dilution blanks and M/5 and M/10 LiOH dilution blanks.

Gray (1), in his study of the action of various inorganic salts on bacteria, showed that apparently some organisms retain their viability unimpaired after incubating 48 hours in media containing as much as 3.3 per cent lithium salts. He also found that all of the organisms tested, with the possible exception of the cholera vibrio, were unaffected in viability by 48 hours of incubation in media containing 0.8 per cent lithium salts.

In the present study a 0.48 per cent or M/5 LiOH solution was the strongest concentration used for dilution blanks. Furthermore, the organisms are in contact with a solution of this strength for only a short interval,[1] before the concentration of lithium salts is greatly decreased through the addition of agar in the pouring of the plate.

Although at this time detailed work with these alkaline blanks has not been entirely completed, sufficient data have been accumulated to show that

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[1] These comparative counts were obtained by following Standard Methods of Milk Analysis (2) which recommend a maximum interval of 15 minutes between preparing the dilution and pouring the agar.

counts obtained using M/5 and M/10 LiOH dilution blanks compare very favorably with parallel counts obtained using distilled water dilution blanks. When a discrepancy occurs it is usually with a powdered acid milk product, the higher counts being obtained when the alkaline blanks are used. This is probably due to the inhibitory effect of a lowered pH in the plates seeded with inocula prepared in distilled water dilution blanks. In some cases this pH is found to be as low as 4.5, which no doubt inhibits colonial development of some of the bacteria present. On the other hand, the pH of the inoculum-medium mixture in the plate is raised to approximately 7.0 when dilutions of the same acid products are prepared with M/5 LiOH dilution blanks.

With a powdered acid milk product whose pH is low, M/5 LiOH dilution blanks seem to give the best results (probably because the acid must first be neutralized before the water-insoluble protein particles can be dissolved), while for a powdered milk product with a higher pH the M/10 LiOH dilution blanks are satisfactory. Excessive shaking of powdered milk dilutions prepared with LiOH solutions should be avoided as the fat has a greater tendency to be churned out in these dilution blanks than with the distilled water dilution blanks.

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STUDIES ON LACTOBACILLUS THERMOPHILUS

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In a review of the literature on the subject of thermophilic and thermoduric bacteria, Robertson (4) stated, "Only one non-spore-forming thermophile, *Lactobacillus thermophilus* Ayres and Johnson, has been isolated." Although thermophilic bacteria have been studied extensively in recent years, it appears that *Lactobacillus thermophilus* has not been encountered since the original isolation. It is therefore pertinent that an additional isolation of the organism be reported and a more complete description presented. As in the original isolation the organism was secured during an investigation of high plate counts with "pin point" colonies from pasteurized milk.

PRELIMINARY OBSERVATIONS

In the routine bacteriological analyses of the Corvallis milk supply, there was noticed a higher bacterial count in the pasteurized milk from one of the local plants, than in the raw milk delivered to the plant. The 1:100 dilution plates on the pasteurized milk were crowded with minute colonies. Daily platings were then made and the plates were incubated at 37° C., with a duplicate set at 50° C. Every sample of the pasteurized milk tested showed thermophilic bacteria which were present in widely varying numbers. Growth at 37° C. was poor in that the colonies were small and sometimes did not appear. At 50° C. the colonies were larger, and by the use of milk powder agar, distinct colonies averaging 0.8 mm. in diameter were obtained. The milk revealed rod forms on direct examination. At the time the preliminary work was being carried on, it was reported from the milk plant that the milk after pasteurization had an acidity of 0.25 per cent calculated as lactic acid, that patrons had complained of a peculiar flavor in the milk, and that a slight odor could be detected in the pasteurizing vat.

DESCRIPTION OF LACTOBACILLUS THERMOPHILUS

A number of cultures of the organism were isolated from colonies on the milk powder agar plates incubated at 50° C. Stock cultures were maintained by making daily transfers in litmus milk, incubating at 50° C. for approximately twenty hours. . The same time and temperature were used throughout this work unless otherwise stated.

Morphology

Form and size. Non-spore-forming rods, averaging 1.0 by 3.5 microns in milk culture. Variations from 2.5 to 6 microns in length are observed. In broth, the cells are somewhat longer. The rods are straight or slightly curved.

Arrangement. Single cells and short chains predominate in milk cultures. In broth, chains occur most frequently, some being very long.

Motility. Non-motile.

Staining reactions. Gram positive, but commonly gram negative in milk culture. The cells are not stained uniformly with methylene blue.

Cultural Characteristics

Agar colonies. On a satisfactory medium (such as milk powder agar), surface colonies are slightly raised, glistening, translucent, circular with a curled edge and vary from 1 to 2 mm. in diameter. Sub-surface colonies are filamentous. Their diameter and density of structure depends upon the medium.

Agar slant. Filiform, glistening, translucent, flat or slightly raised growth on milk powder agar.

Broth. Slight growth in plain broth. In broth rich in peptone, good growth takes place. No surface growth, no sediment, moderate clouding.

Litmus milk. Acid with partial reduction of the indicator.

Biochemical Features

Indol—not produced.

Nitrates—not reduced.

Action on carbohydrates and alcohols. Glucose, galactose, levulose, mannose, lactose, maltose, sucrose, raffinose, soluble starch and dextrin fermented with the production of acid, but no gas. Arabinose, xylose, glycerol, rhamnose, salicin, insulin, and mannitol not fermented.

Oxygen relationships. Organism facultative; in agar-shake cultures the best development of colonies takes place near the surface of the medium.

Temperature relationships. Slight or no growth at 30° C. Weak growth at 35° C. (On plates at this temperature only a small part of the total inoculated will grow compared with duplicates at 50° C.) Optimum 50° to 60° C. Maximum at approximately 62° C. A milk culture is rendered sterile on heating at 70° C. for thirty minutes.

Total and Volatile Acid

The total and volatile acids were determined in milk cultures of the organism after incubation for 48 hours at 55° C. The total acid was determined by titrating 10-gram samples with 0.1 N. NaOH using phenolphthalein as an indicator, and calculating the acidity as lactic acid. A large number of determinations have all shown from 0.35 to 0.42 per cent. The volatile acid was determined by steam distillation of a 250-gram sample of the milk, after acidifying with 15 ml. of N. H_2SO_4. One liter of distillate was collected and titrated with 0.1 N. NaOH using phenolphthalein as an indicator. The following milliliters of 0.1 N. NaOH, 14.5, 15.6, 10.9 and 13.0 indicate that comparatively little volatile acid was produced, as the figures for sterile milk treated in the same manner are from 3 to 6.

Determination of the Non-Volatile Acid

It is apparent that the non-volatile acid makes up most of the total acid. Zinc salts were prepared from the residues remaining after the steam distillations, using the method by Hammer (3). The results obtained in the study of these salts show that the non-volatile acid formed is largely or entirely *d* lactic acid.

Comment upon the Description

The organism described is practically identical to *Lactobacillus thermophilus* Ayres and Johnson, and may be considered as the same species. From the additional characteristics recorded in the description, it would seem that the possible similarity between *Microbacterium lacticum* Orla-Jensen and *Lactobacillus thermophilus* Ayres and Johnson, as was suggested by Robertson (5), does not hold true.

FACTORS AFFECTING THE GROWTH OF LACTOBACILLUS THERMOPHILUS

The conditions of the Standard Plate Count are not particularly favorable for the growth of *Lactobacillus thermophilus.* The temperature of 37° C. is near the minimum for the organism and at two or three degrees lower (temperatures which exist in many routine incubators) normal development does not take place. Secondly, the organism does not grow in slightly acid media. With other conditions favorable, a pH of 6.0 in agar media, determined colorimetrically, prohibits growth. Many batches of agar used in routine work, but not carefully adjusted to a pH of 6.2 or above, have failed to grow the organism. Thirdly, standard nutrient agar is not a very satisfactory medium for the growth of *Lactobacillus thermophilus.* Surface colonies do not appear, nor can appreciable surface growth be induced by streaking (this is characteristic of many lactobacilli), although on a more nutritive medium surface colonies do appear. The sub-surface colonies on standard nutrient agar are filamentous, and of very thin veil-like structure averaging 0.4 mm. in diameter, and though not "pin-point" in size, are not readily seen in some cases.

A preliminary study was made of the nutritive requirements of *Lactobacillus thermophilus* in regard to the type and amount of peptone. By substituting Bacto-Proteose peptone in standard nutrient agar, in place of Bacto-Peptone, in the usual 0.5 per cent concentration, luxuriant growth is obtained. Surface and sub-surface colonies are from one to two mm. in diameter. Almost as good growth can be obtained by increasing the concentration of Bacto-Peptone to two or three per cent. In regard to the minimal peptone concentrations, 0.1 per cent Bacto-Proteose peptone in washed agar without beef-extract supports the growth of distinct colonies, whereas 0.3 per cent Bacto-Peptone is required to grow visible colonies. Here again, any considerable variation from the standard methods formula might not allow growth of *Lactobacillus thermophilus*. The required nutritive substance is present in peptone, particularly proteose-peptone. The organism is not saccharophilic as are the thermo-tolerant "pin-point" streptococci. The addition of diluted sterile milk to an agar plate culture increases the colony size of *Lactobacillus thermophilus*, but sterile lactose has little or no effect.

VIABILITY OF LACTOBACILLUS THERMOPHILUS

In maintaining stock cultures, it was found that cultures of the organism rapidly became sterile. Milk cultures which had been incubated at 50° C. for 12 to 24 hours and then held at room temperature were sterile in from four to ten days. Cultures containing calcium carbonate remain viable only somewhere longer. Broth cultures and colonies are usually sterile in a week or less. Storing cultures at ice-box temperatures lengthens the period of viability.

THE DEVELOPMENT OF LACTOBACILLUS THERMOPHILUS IN THE PASTEURIZING PLANT

The organism was regularly present in the pasteurized milk from one plant during a period of several weeks. The raw milk received at the plant did not reveal the organism either by plating or direct microscopical examinations. As the plant equipment has often been reported as a source of thermophiles, this possibility was investigated. The pasteurizing vats of the Burrell Spray type were in good condition and attempts to find the organism in the cleaned vats just before use were unsuccessful. Swabbings from the sides and corners of the vat as well as drip water in the bottom did not contain thermophilic bacteria. The failure to isolate the organism in the raw milk and in the equipment seemed inconsistent with the daily appearance of large numbers of the organism in the pasteurized milk. However, a check-up on the plant operations did explain the problem. The milk was being standardized before pasteurization to the desired butterfat content with the pasteurized cream of the previous day. It was a case of daily inoculation as the data in the table show.

Thermophilic bacteria in milk shown by platings at 50° C.

Mixed raw milk in vat before heating	0 in 0.01 cc.
Raw milk after addition of cream	40,000 per cc.
The same, after heating to 143° F.	60,000 per cc.
" , after holding 45 minutes	100,000* per cc.
The cream used to standardize	1,000,000* per cc.

* Approximate numbers due to low dilutions used.

Since discontinuing the addition of pasteurized cream to standardize the milk, weekly platings of the pasteurized milk have been continuously free from *Lactobacillus thermophilus.* The trouble was therefore due to re-pasteurization, often recognized as a source of high counts of thermophilic bacteria. The unusually large numbers in the cream, which gave a sizable inoculation to each vat of milk in the standardizing process, can be explained. The cream was obtained by separating the pasteurized milk at the end of each day's run, during which the vats were in operation from four to six hours, and not sterilized between batches. Pasteurization was performed at 143° F. for 45-minute periods. A further factor favorable to the development of the organism was the limited capacity of the separator which delayed the eventual cooling of the warm milk from the last vat. As Ayres and Johnson (1) have pointed out, the organism is readily killed by efficient steaming of the equipment, and for this reason equipment infections of this organism are not so likely as in the case of the spore-forming thermophilic bacteria.

THE SOURCE OF LACTOBACILLUS THERMOPHILUS

The organism was not isolated from the raw milk delivered to the plant. Six weekly platings from each of twenty-five shippers' milk were consistently free from the organism in the 0.01 cc. amounts tested. Attempts to stimulate growth by a prior four-hour incubation of the milk at 50° C. were not successful. More recently, four attempts to isolate the organism from the pasteurized milk and also from the raw milk from seven shippers to the Iowa State College pasteurizing plant, were unsuccessful. This evidence agrees with the statement of Ayres and Johnson, "It seems probable that the organism may be generally present in raw milk, but in small numbers, which may increase under suitable conditions. The source of the organism is therefore not clear.'' These authors did isolate the organism from raw milk but their data show 10 shippers' milk free from thermophiles in the amounts tested, and that the high counts were due to a plant infection.

A similar organism, probably the same species, as judged by colony formation, morphology, and heat resistance, was recently the cause of trouble in another Oregon pasteurizing plant and it was traced to the milk from one shipper.

A close scrutiny of the bacteriological analyses of the milk supply of Corvallis for more than a year and of a much greater number of analyses of the Portland milk supply during the entire years 1926–27, has failed to reveal any outbreaks of thermophilic bacteria as shown by the Standard Plate Count, other than in the two cases mentioned. During this time, more than a hundred cases of high counts in pasteurized milk showing typical ''pin-point'' colonies have been investigated. In almost every case streptococci were the cause of the trouble. Studies on these organisms in regard to morphology, heat resistance, poor growth on standard nutrient agar and their saccharophilic nature have agreed with the results of Fay (2). It is a simple matter to differentiate the filamentous colony of *Lactobacillus thermophilus* from the more typical lens-shaped ''pin-point'' colonies characteristic of the thermo-tolerant streptococci.

As a by-product of this work it appears that under the conditions of the Standard Plate Count, spore-forming thermophiles have not been the cause of high-counts in the very large number of routine milk analyses observed. Although these organisms are wide-spread in nature they are not common in raw milk in large numbers as hundreds of platings on raw milk at 50° C. have revealed few and usually no thermophilic bacteria of any kind in the 0.01 cc. amount tested.

SIGNIFICANCE OF LACTOBACILLUS THERMOPHILUS

In the present investigation the organism appeared as a result of repasteurization, which is prohibited by most city milk ordinances. In the work of Ayres and Johnson, failure to sterilize the pasteurizing vat resulted in high counts of the organism. Hence, in these cases the presence of the organism has been indicative of faulty plant procedure. Cultures of the organism have been consumed without harmful results.

SUMMARY

An additional isolation of *Lactobacillus thermophilus* is reported. The organism agrees in almost every respect with the original description. Additional characteristics are recorded. The organism grows poorly on the nutrient agar used in routine milk platings, and the indistinct filamentous

colony may be "pin-point" in size. The colony can easily be distinguished from the lens-shaped "pin-point" colonies of the thermoduric streptococci. Much better growth is obtained when Bacto-Proteose peptone is substituted for ordinary peptone.

As in the original isolation *Lactobacillus thermophilus* is found to be the causative organism of high bacterial counts in a pasteurized milk supply. It would appear that the organism is not of common occurrence in milk. The particular growth requirements and the relatively short period of viability characteristic of the organism may also explain why it has not been isolated more frequently.

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(3) Hammer, B. W. The type of lactic acid produced by starters and by organisms isolated from them. Res. Bull. Ia. Agr. Expt. Sta. 65. 1920.

(4) Robertson, A. H. Thermophilic and thermoduric microorganisms with special reference to species isolated from milk. I. Review of literature. New York State Agr. Expt. Sta. Tech. Bull. 130. 1927.

(5) Robertson, A. H. Thermophilic and thermoduric microorganisms with especial reference to species isolated from milk. III. Description of the non-spore-forming, thermoduric organisms isolated. New York State Agr. Expt. Sta. Tech. Bull. 131. 1927.

SOME OBSERVATIONS ON THE EFFECT OF TEMPERATURE OF PASTEURIZATION ON THE ACID COAGULATION OF SKIMMILK*

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In studying the effect of temperature of pasteurization on the manufacture of cottage cheese, it was noticed that coagulation by culture-developed lactic acid occurred noticeably sooner when the skimmilk had been pasteurized for 30 minutes at 85°C. (185°F.) than for the same period at 62.8°C. (145°F.). This observation led to some conjecture as to the relation of temperature of pasteurization to the phenomenon of acid coagulation. An experiment was therefore planned to determine the effect of temperature of pasteurization of skimmilk on the time required for coagulation and on the curd tension, total acid developed, and H-ion concentration, each at the time of coagulation.

For this study mixed herd milk was separated and the skimmilk divided into six 600 cc. portions. Five of these were pasteurized in liter pyrex beakers at 62.8°C. (145°F.), 68.3°C. (155°F.), 73.9°C. (165°F.), 79.4°C. (175°F.), and 85°C. (185°F), respectively, each for a holding period of 30 minutes. Pasteurization was accomplished with a water bath heated by a gas flame. Each beaker was constantly watched during the heating and holding periods so that the holding temperature was controlled within one degree of that stated. The milks were cooled as soon as the holding period ended and were stored over night in an ice-box. The sixth sample was not heated. On the following morning duplicate samples of 200 grams from each were weighed into 250 cc. pyrex beakers, eight grams of active lactic culture added, all samples warmed to 30°C. in a water bath, then set in a constant-temperature oven at 30°C. A Hill[1] curd knife was placed in each beaker. In every case coagulation was allowed to progress until a jelly-like curd was evident. The curd tension was then determined by attaching a sensitive spring balance to the curd knife and drawing it slowly through the curd. The curd tensions recorded represent in each case the total pull in grams less the weight of the knife. Acidity was determined by titration with tenth-normal sodium hydroxide to a faint

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[1] HILL, R. L. The physical curd character of milk and its relationship to the digestibility and food value of milk for infants. Utah Agr. Exp. Sta. Bul. 207. 1928.

pink with phenolphthalein, and calculated as per cent lactic acid. Hydrogen-ion concentration was determined by means of a Leeds and Northrup laboratory potentiometer using a hydrogen electrode. Five complete trials were conducted.

Effect on curd tension—The effects of the various temperatures on the curd tension at coagulation are shown in figure 1. This curve was drawn free-hand through the arithmetic means of the tensions found for each temperature. The scatter diagram of the five trials is also shown.

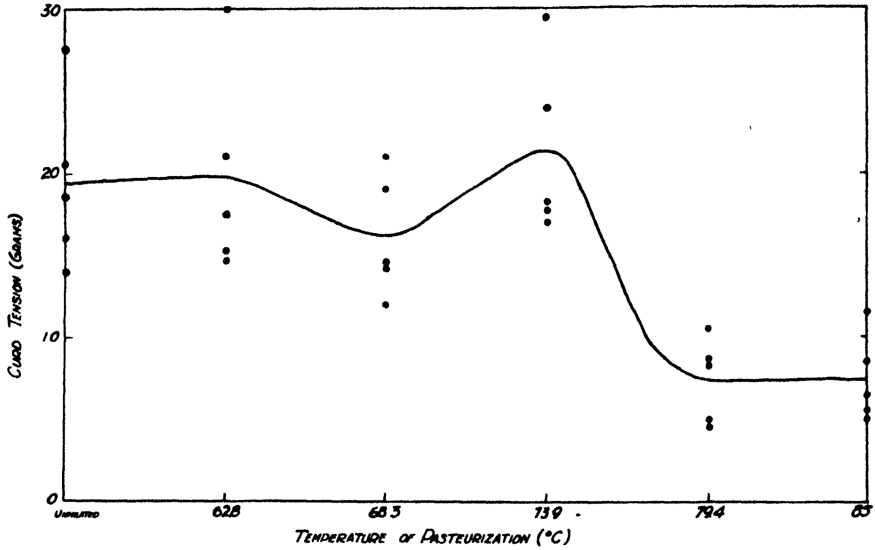

Fig. 1. Relation of Temperature of Pasteurization to Curd Tension of Skimmilk After Curdling.

The significant effect shown here is that curd tension of the acid coagulum is lower for skimmilks pasteurized at 79.4° and 85°C. than for those pasteurized at the lower temperatures or not heated. Variations in the curve at the lower temperatures used probably are not significant. Hill[1] found that duplicate determinations made by the use of his knives and balance sometimes varied as much as 10 grams, and in two cases such variations occurred in this work. It is likely that the variations in these data were due to experimental errors resulting from the use of a method not adaptable to precise determinations.

The fact that high-temperature pasteurization causes, on acid coagulation, a softer curd than results from the use of lower temperatures of pasteurization has often been observed. This effect has particular application in the manufacture of culture buttermilk and cottage cheese. The acid coagulum from skimmilk pasteurized at 73.9°C. or higher for a thirty-minute holding period appears to consist of smaller particles than the coagulum from skimmilks pasteurized at lower temperatures or not heated, and the curd breaks and shatters more readily on stirring. In the manufacture of culture buttermilk this property is desirable but in cottage cheese making it is undesirable, being responsible for the breaking of curd flakes on stirring during the "cooking" process.

Effect on H-ion concentration and total acidity—The relationship of temperature of pasteurization to H-ion concentration and total acidity are shown in figure 2. Total acidity at coagulation was higher for skimmilk pasteurized at 62.8°C. than for unheated skimmilk, but showed a gradual and uniform decrease as the temerature of pasteurization was increased. H-ion concentration at coagulation was the same for the unheated skimmilk and for that pasteurized at 62.8°C., but showed a gradual decrease as the higher pasteurization temperatures were used. One additional sample was heated to 92°C. and held for 30 minutes. When coagulated by the method used for the other samples its total acidity was 0.65 per cent and its H-ion concentration 0.93×10^{-5}, both of which were lower than any of the results at 85°C. The pH values are shown in the accompanying table.

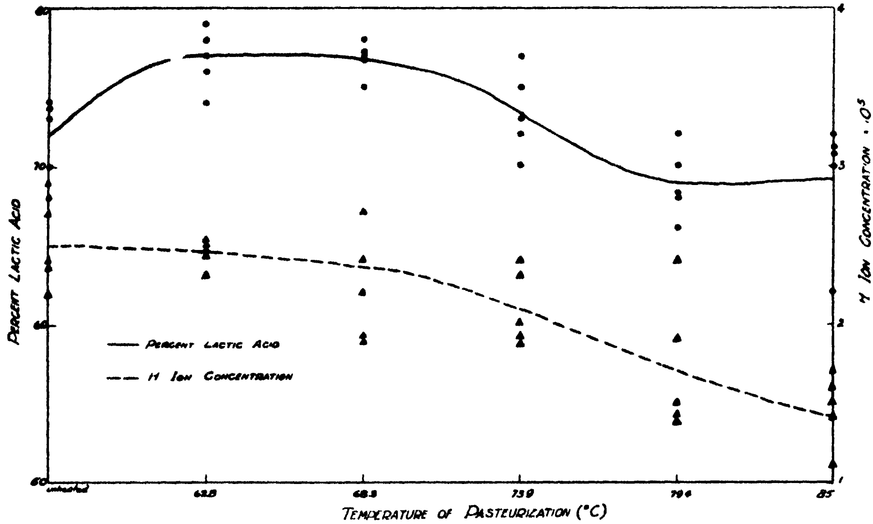

FIG. 2. RELATION OF TEMPERATURE OF PASTEURIZATION TO ACIDITY AND H-ION CONCENTRATION AFTER CURDLING.

There is evidence here that heat acts upon casein so that its coagulation may be brought about with less acid than is necessary in unheated milk. Whittier and Benton[2] have shown that continued boiling of whole milk causes a gradual increase in acidity and H-ion concentration. In their studies coagulation occurred at a pH of 5.6. The fact that coagulation

[2] WHITTIER, E. O. and BENTON, ANNE G. The formation of acid in milk by heating. JOUR. DAIRY SCI. 10: 126. 1927.

occurred at pH 5.6 when the temperature was at boiling is not surprising, as it is generally understood that heating milk of relatively low acid content brings about coagulation; but data herein presented show that previous heating followed by coagulation at a relatively low temperature produced the same apparent effect, though somewhat smaller in magnitude. These facts indicate that heat applied to milk produces two effects; *i.e.*, it produces acid (2), and it affects the casein so that acid precipitation occurs at a H-ion concentration lower than the iso-electric point of normal unheated milk by an amount that is roughly proportional to the intensity of the heat treatment.

The relation of temperature of pasteurization to pH at coagulation

TEMPERATURE TREATMENT	PH (AVE. OF FIVE TRIALS)
°C.	
Unheated	4.598
62.8	4.607
68.3	4.611
73.9	4.673
79.4	4.769
85	4 839
*92	*5.027

* Single trial.

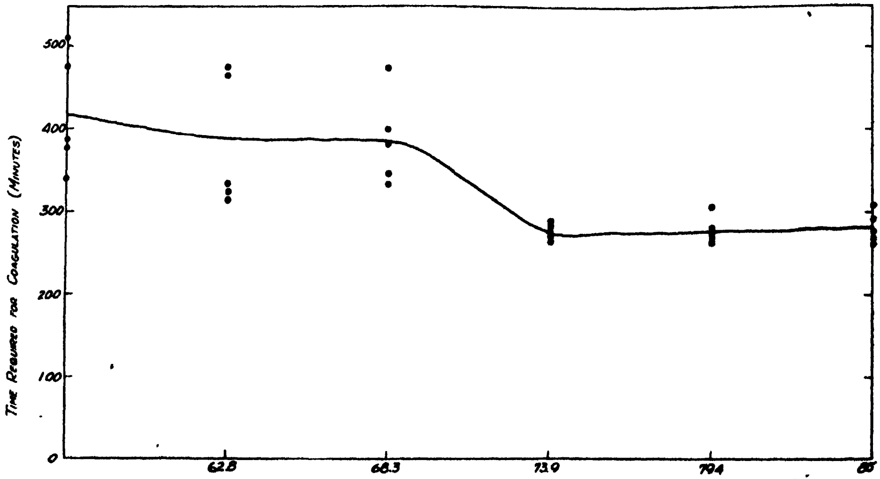

FIG. 3. RELATION OF TEMPERATURE OF PASTEURIZATION TO TIME REQUIRED FOR CURDLING.

Effect on time required for coagulation—The effect of the various temperatures on the time required for coagulation is shown by figure 3. The curve was drawn free-hand through the arithmetic averages of the time periods required for coagulation at each temperature. A rather sharp reduction in the time required for coagulation occurred between the skim milks pasteurized at 68.3° and those pasteurized at 73.9°C. Little change occurred between the times required for the group including the unheated skim milks and those pasteurized at 62.8° and 68.3°C., while still less change occurred in the group including the skim milk pasteurized at 73.9°, 79.4°, and 85°C., respectively.

This effect is explained, in part, by the fact that high-temperature pasteurization makes it possible to precipitate casein with less acid than is needed for unheated skimmilk, or skimmilk pasteurized at lower temperatures, as already pointed out. However, the differences in amount of acid required as the higher temperatures were used were relatively small and their change from low to high-temperature treatment produced a uniformly sloping curve not comparable to the curve of the time periods required for coagulation due to the sharp change of the latter between the temperature at 68.3° and 73.9°C. None of the data from these trials explain this irregularity of the time curve.

The time factor in acid coagulation has some significance in the manufacture of culture buttermilk and cottage cheese. The coagulation time is markedly reduced by increases in the intensity of previous heat treatment.

SUMMARY

Increasing the temperature of pasteurization of skimmilk was found to have several significant effects on its coagulation by lactic culture. The firmness of the coagulum, or the curd tension, at the time a jelly-like curd first formed, was roughly twice as great for raw skimmilk and skimmilks pasteurized at 62.8°, 68.3°, 73.9°C. as for those pasteurized at 79.4° and 85°C.

Increase in temperature of pasteurization reduced the H-ion concentration and also the amount of acid present at the time of coagulation. This decrease was slight up to 68.3°, above which the change was more marked with increasing temperature.

The time required for coagulation under uniform setting conditions was little changed by pasteurization at temperatures up to 68.3°, but was reduced materially at 73.9°C., beyond which there was little further change.

INFLUENCE OF ARSENICAL DIPPING ON YIELD OF MILK BY DAIRY COWS

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INTRODUCTION

Since the advent of tick eradication through cooperation of federal and state authorities in eradicating the cattle fever tick, *Margaropus annulatus,* the immediate effect upon milk flow has been questioned. An appreciation of the insignificant effect of the arsenical dipping on milk yield should stimulate efforts towards complete eradication of this tick in the remainder of this, and other countries. For interpretation of experimental work in progress at the Florida Agricultural Experiment Station, it was necessary to measure the magnitude of the effect upon milk production. The dairy cows in the station herd were only very slightly infested with fever ticks, and hence no measure of comparison is intended in this paper between tick-infested and tick-free conditions.

REVIEW OF LITERATURE

Three investigations of the effects of the use of arsenical solutions, as employed in tick eradication, have been reported. Each of these was conducted at about the same time with selected groups of cows in single lactation periods. Scott,[1] at the Florida station, dipped nine cows for six consecutive times (54 cow-dips) at 14-day intervals. Milk records of five days preceding and succeeding the dipping were compared, and a total decrease of 8.68 per cent in milk yield noted.

McClain[2] reported 10 cows dipped seven times, or 70 cow-dips, in South Carolina with an average decline of 10.6 per cent in milk production in the two-day period after dipping. The first four dips caused a decline of 14.8 per cent milk and 8.9 per cent in butterfat production. The last three dips caused a loss of only 1.9 per cent in milk yield and an increase of 10.6 per cent in butterfat yield. The last three dips are hardly significant, since the decrease in milk yield was approximately the same as expected from advance in lactation.

Received for publication January 18, 1932.

[1] Scott, J. M. How dipping affected the milk flow. Fla. Agr. Exp. Sta. Ann. Rept. 1915: xx–xxi.

[2] McClain, J. H. Eradication of the cattle tick necessary for profitable dairying. U. S. D. A. F. Bul. 639: 1–4. 1914.

A question arose as to whether the decrease in production was caused by the shock of dipping, or by arsenical absorption. To determine this point, Woodward, Turner and Curtice[3] obtained records from 20 cows which were sprayed rather than dipped at four consecutive bi-weekly intervals. By comparing milk yields for three preceding days with those of the five days after spraying, they noted an average reduction of 6.1 per cent in production. The cows required from three to five days to recover from the effect of the spray.

METHODS OF INVESTIGATION

The systematic tick eradication campaign in Alachua county was conducted by Federal and State authorities in accordance with the Florida Laws, Rules and Regulations governing tick eradication. The standard arsenical solution was used which contained from 0.18 to 0.20 per cent arsenious oxide (As_2O_3). All cattle in the county were dipped at 14-day intervals from March, 1930, to May, 1931, inclusive, with the exception of the months of January, February and March, 1931, when a 28-day interval was allowed. A total of 29 dip days was entailed with the Experiment Station herd during the campaign in this area. However, even before the campaign, ticks rarely could be found on any of the cattle in the herd.

The data presented herein were obtained from the milking cows in the Experiment Station dairy herd. These cows were driven one-half mile down a lane to the vat, and dipped shortly after the morning milking. They were turned on pasture during the day.

Within the province of this paper, a day's milk constitutes the total milk obtained at the afternoon and following morning milkings. The cows were milked at 5:00 A. M. and 3:00 P. M. daily, except on Sundays when the afternoon milking started at 2:30 P. M. with all but the cows on feeding experiment. Every milking of the cows in the station herd was used in the tabulations.

Since Alachua County, Florida, is located in the southern portion of the temperate zone close to large bodies of water, the climatic conditions differ from those of other regions. Winter is here defined as the interval between the average date of first killing frost in autumn and the last frost in spring. Tabulations of mean temperatures and of average rainfall by months (from Florida Agr. Exp. Sta. Bul. 200) were used to delineate "summer,"—the months of June to September inclusive. Spring and autumn were thus automatically defined. In addition to average climatological data upon which seasons are here defined, the actual rainfall and mean temperatures experienced during the eradication campaign, are shown in table 1.

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[3] Woodward, T. E., W. F. Turner and C. Curtice. Effect of the cattle tick upon milk production of dairy cows. U. S. D. A. Bul. 147: 14–16. 1915.

TABLE 1

The 17-year average and the 1930–1931 actual climatic conditions as related to seasons in Alachua County, Florida

MONTH	AVERAGE DATES OF KILLING FROSTS		MEAN TEMPERATURES						SEASONS
			MINIMUM		MAXIMUM		RAINFALL		
	Average	Actual	Average	Actual	Average	Actual	Average	Actual	
			degrees	*degrees*	*degrees*	*degrees*	*inches*	*inches*	
March (1930)	2nd*	5th	51.9	48.5	75.5	67.8	3.21	6.49	Spring
April			57.3	57.7	81.3	80.2	2.21	2.12	"
May			63.3	63.4	86.6	89.2	3.11	4.99	"
June			68.8	68.3	89.7	85.9	6.57	12.03	Summer
July			70.7	71.6	90.1	92.4	7.32	3.10	"
August			70.9	69.0	90.7	90.9	6.76	3.40	"
September			69.0	70.9	88.9	90.7	5.65	8.64	"
October			62.0	57.5	82.7	80.1	2.84	1.08	Autumn
November	27**	29th	50.9	51.2	74.0	72.4	2.04	1.68	"
December			47.4	42.2	68.9	60.5	3.22	4.90	Winter
January (1931)			47.1	42.4	68.5	65.1	3.31	4.14	"
February			47.0	45.1	69.8	67.5	2.87	2.72	"
March	2nd*	5th	51.9	46.7	75.5	69.5	3.31	5.05	Spring
April			57.3	55.0	81.3	78.4	2.21	2.83	"
May			63.3	61.1	86.6	85.4	3.11	6.11	"

* Last killing frost in spring.

** First killing frost in autumn.

PRESENTATION OF DATA

The seasonal distribution of dippings, when the seasons were defined as outlined in table 1, was: spring, 7; summer, 13; autumn, 4, and winter, 5. Milk yields for the entire cow herd during the tick eradication campaign have been tabulated by seasons. Average daily milk yields, weighted for the entire 826 cow-dips, were calculated. With cows freshening and going dry in approximately equal numbers thruout the period, it was theoretically possible to neglect the decline in production due to the advance in lactation period as a factor affecting daily milk yield.

The decline in milk production for each day, and for the week after dipping for the different seasons and the entire tick eradication period, is presented in tables 2 and 3.

TABLE 2

Average daily milk yield per cow, by seasons, during the tick eradication campaign

"DAYS"*	SEASON AND NUMBER OF DIPPINGS				WEIGHTED AVERAGE
	Spring 7	Summer 13	Autumn 4	Winter 5	
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
Before dipping					
7	15.88	17.38	16.60	13.84	16.29
6	15.88	17.15	16.97	13.82	16.26
5	15.93	17.47	16.89	14.10	16.44
4	15.90	17.67	16.67	13.88	16.46
3	15.75	17.40	17.00	13.86	16.44
2	16.23	17.52	16.54	13.36	16.29
1**	16.48	17.30	16.70	13.60	15.88
After dipping					
1	14.74	16.25	15.99	12.82	15.28
2	16.03	16.67	16.02	13.18	15.67
3	16.34	17.08	16.57	13.28	16.04
4	16.17	17.14	16.84	13.50	16.27
5	15.98	17.28	16.32	13.42	16.24
6	16.00	17.32	16.57	13.62	16.29
7**	16.34	16.95	15.79	13.44	16.06

* A "day" constitutes the P. M. and following A. M. milkings.

** Sunday afternoon milking was one-half hour earlier, except with cows on feeding experiment.

DISCUSSION OF RESULTS

With these 826 cow-dips through fifteen months of dipping under state and federal regulations and Florida climatic conditions, it has been pos-

TABLE 3

Percentage milk yield on seven days following dipping, using the average of the preceding seven days as 100 per cent

"DAY"	SEASON				WEIGHTED AVERAGE
	Spring	Summer	Autumn	Winter	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	92.08	93.32	95.37	93.03	93.78
2	100.14	95.73	95.54	95.65	96.17
3	102.08	98.09	98.82	96.37	98.44
4	101.02	98.43	100.43	97.97	99.85
5	99.83	99.24	97.33	97.39	99.67
6	99.96	99.47	98.82	98.84	99.97
7	102.08	97.34	94.17	97.53	98.56
Average	99.58	97.37	97.21	96.68	98.06

sible to verify earlier work and to show a decline in milk production lasting from three to four days after dipping. However, the percentage loss observed under these conditions was considerably lower (1.94 per cent, comparing previous and succeeding weeks' milk yields) than given in earlier reports. Since previous studies were all made with cows in single lactation periods, the decline in production given would include the normal decline due to the advance in lactation as well as that due to dipping. In this study, cows freshened and went dry in approximately equal numbers during the entire period, which would theoretically eliminate the effects of advance in lactation, so that the declines observed can be attributed almost completely to the effects of the dipping.

Apparent recovery was most rapid in the spring, followed in turn by autumn, summer, and winter. More rapid recovery of milk flow during the spring months may possibly be due to the stimulating influence of early pasture. Slower recovery, requiring four to five days, in the winter is associated with the shock of dipping and the chilling of the animal, together with a lack of green pasture. Declines for the first four days after dipping for the entire period, were 6.22, 3.83, 1.56 and 0.15 per cent respectively. No tolerance to arsenic absorption was developed, as decline in milk production occurred after each dipping during the whole eradication campaign.

The largest decline in milk yield was noted on the second milking following the dip, and appeared to be caused by slow absorption of arsenic through the skin, rather than by the immediate shock and excitement of dipping. This is confirmed by the loss of milk observed by Woodward and associates, who sprayed the cows with arsenical solution, rather than dipped them. They noted a 6.1 per cent decrease in milk yield and a three to five day period of recovery. That the chilling and excitement of dipping are small factors, except in cold weather, is indicated from this work.

SUMMARY AND CONCLUSIONS

A total shrinkage in milk flow of only 1.94 per cent, comparing succeeding with previous week's milk yield, was observed in a tabulation of 826 cow-dips at the Florida station during the tick eradication campaign in Alachua county. The greatest decrease in production was in the second milking after the cows were dipped, indicating that the effect was produced by arsenic absorption rather than by the excitement and shock of dipping. No tolerance to the arsenic was developed. The effect of dipping was greatest in the winter, followed by autumn, summer, and spring, indicating that low temperatures emphasize the effect of dipping. Shrinkage in milk yield was 6.22, 3.83, 1.56 and 0.15 per cent consecutively for the first four days after dipping. The decline in average daily milk yield due to advance in lactation, was practically eliminated in these tabulations by the fact that cows freshened and were turned dry in approximately equal numbers throughout the campaign.

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THE VIABILITY OF LACTOBACILLUS ACIDOPHILUS AS AFFECTED BY FREEZING IN A SHERBET MIXTURE*

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Lactobacillus acidophilus milk is unpalatable to many persons. Some object to the cooked flavor brought about by the high pasteurization temperature used in its preparation, while others dislike the flavor imparted during the process of fermentation. Partially to overcome these objections and partially for exploitation purposes, numerous attempts have been made to market the organism in the form of tablets, powders, semi-solid candy-coated preparations and broth cultures. Numerous investigators have shown the tablets, powders and candy-coated preparations to be of little value and usually worthless as conveyors of viable organisms. In many instances, broth cultures have been found to be devoid of or extremely low in number of viable organisms by the time they reached the consumer. For instance, Black (1) made a quantitative analysis of a broth culture prepared for distribution by a commercial laboratory. This preparation was labeled to contain 200,000,000 viable organisms at the time of manufacture and 100,000,000 viable organisms at the expiration date one month later. He found a maximum plate count of 48,000,000 per cc. three days after the date of manufacture. Unpublished observations made by us have resulted in similar findings. Of the preparations employed as conveyors of *L. acidophilus* organisms, milk cultures have shown the highest average bacterial counts. All investigators, however, do not agree as to the most desirable method of maintaining the viability of these organisms in milk cultures.

REVIEW OF LITERATURE

Low temperatures improve the palatability of *L. acidophilus* milk. However, it has been the prevailing opinion among bacteriologists that the number of viable *L. acidophilus* organisms in milk is reduced more rapidly when the product is stored at refrigerator temperature than when stored at room temperature. Kopeloff (2), as well as Reichart and Davis (3), have presented data to uphold this belief. Trammal and Hastings (4), found the organisms to remain viable for a longer period of time at 10°C. to 13°C. than at 22°C. Black (1) working with a number of strains of both *L. acid-*

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ophilus and *L. bulgaricus* found a lower reduction in numbers at refrigerator temperature, 9°C. or even 0°C., than at 37°C. He noted little difference in the viability of *L. acidophilus* and *L. bulgaricus* in milk kept for several days at refrigerator and room temperatures. Black and Harris (5) found a storage temperature of 9°C. to result in a smaller reduction of viable organisms than storage at room temperature. They found the largest number of organisms present at low acidities and the greatest destruction of organisms in the most acid samples. From these results, they concluded that the reaction of the stored culture is a more important factor in decreasing the number of viable organisms than the storage temperature.

In a recent study, Kulp (6) found the continued life of *L. acidophilus* to be dependent upon two factors; namely: the initial content of acid or some related metabolic substance and the temperature of storage. He found that commercial *L. acidophilus* milk of good quality and containing a satisfactory number of organisms which would remain viable for two days to one week, might be stored at 5°C., provided the initial acidity did not exceed 0.65 per cent. To insure viability for periods longer than one week, he found it necessary to reduce contamination to a minimum, to keep down acidity and to maintain the storage temperature between 12°C. and 16°C., the temperature for optimum viability being 16°C. with an initial acidity of 0.65 per cent. Myers (7) recently developed a sweet milk product carrying large numbers of viable *L. acidophilus* organisms to which he gave the name unfermented *L. acidophilus* milk. The *L. acidophilus* organisms are grown in a sterilized medium and freed from it without contamination. They are then suspended in pasteurized milk in numbers comparable to those found in a good cultured acidophilus milk. He found the death rate of *L. acidophilus* in such a preparation to be very low when stored at 2°C. to 5°C. for a period of four to five days and attributed this low death rate to the low acid content of the product.

EXPERIMENTAL

To increase the palatability of *L. acidophilus* milk, it was sweetened, flavored, and frozen into a sherbet. In addition to a high sugar concentration, the organisms were exposed to extremely low temperatures while the product was in storage. Since the therapeutic benefit derived from any *L. acidophilus* product is dependent upon the numbers of viable organisms at the time of consumption, a study was made of the viable organisms remaining in the sherbet at intervals throughout a seven-day storage period.

Each culture of *L. acidophilus* used in this study was transferred daily for a period of several days prior to use. From this actively growing culture a transfer was made into a volume of sterile skim milk sufficient to prepare several sherbet mixes. After incubation at 37°C., usually for a period of twenty-four hours, the necessary ingredients were added and the mixture frozen into a sherbet. The general composition of the mixes was as follows: sugar 32 per cent, *L. acidophilus* milk 48 to 68 per cent, flavoring material 0 to 20 per cent. In a few instances, some variation from the above was made in the sugar content. The flavoring materials used were fresh lemon and orange juices, crushed pineapple, citric acid, lactic acid, grape juice, and artificial walnut and coffee extracts. The freezing process was carried out in a two-quart freezer which previously had been sterilized in the autoclave. Plate counts using Bacto-dehydrated galactose whey agar were made of the cultured milk, the freshly prepared mix, immediately after freezing and after storage periods of 1, 2, 3, 5 and 7 days. Acid determinations were made of both the cultured milk and the prepared mix. The storage temperatures varied from -17°C. to -25°C. A total of 28 sherbets was studied. The results are presented in table 1.

DISCUSSION

The results presented in table 1 show a variation in the resistance of the strains of *L. acidophilus* to the conditions under which they were subjected when frozen in a sherbet mixture. Strain A showed the most rapid reduction in numbers of viable organisms. After five days of storage at temperatures below -17°C. the viable organisms in sherbets carrying this strain were reduced to only a few million per cc., while under similar conditions several hundred millions of viable organisms per cc. were present in the sherbets carrying the other strains of *L. acidophilus*. Pronounced reductions of organisms of strain A were evident after one day of storage. There were fewer initial organisms in cultures of this strain than in cultures of other strains. Since all of the cultures were incubated under similar conditions prior to being frozen into sherbets, it is apparent that the low initial numbers of organisms of strain A and their inability to remain viable during storage were due to inherent characteristics of the strain.

A variation in viability was found to occur in cultures of the same strain. This variation was very pronounced in cultures of strain O represented by sherbets 12 and 14. Sherbet 12 having an acid content of 0.75 per cent and an initial count of 1,670 million organisms per cc. contained 160 million viable organisms per cc. after a storage period of seven days, while sherbet 14 with an initial acid content of 0.65 per cent and an initial count of 900 million organisms per cc. contained 700 million viable organisms per cc. after a similar storage period. Significant variations occurred in cultures represented by sherbets 2 and 8, 12 and 15, 20 and 23. A study of these sherbets, in which two different cultures of the same strain are represented, showed no correlation between either the initial acidity of the culture or the acidity of the mix and the numbers of viable organisms re-

TABLE 1

Viability of Lactobacillus Acidophilus in Sherbets

NO. OF SHERBET	STRAIN	CULTURE	FLAVOR	PER CENT SUGAR	PER CENT ACID		BACTERIAL COUNT IN MILLIONS PER CC.							
										Time in Storage				
					Culture	Mix	Culture	Mix	After freezing	1 day	2 days	3 days	5 days	7 days
1	K	1	None	32	0.57	0.47	1010	650	640	840	735	750	700	—
2	K	1	Lactic acid	32	0.57	1.62	1010	580	—	750	545	550	500	—
3	K	2	Lactic acid	32	0.40	1.90	830	640	400	360	315	290	240	—
4	K	2	None	32	0.40	0.36	830	700	690	600	510	500	480	—
5	K	3	None	35	1.15	0.88	3040	2300	1720	1530	—	1550	—	1400
6	K	3	None	20	1.15	0.95	3040	2080	1880	1800	—	1860	—	1600
7	K	4	Pineapple-lemon	18	1.02	1.17	1670	1470	1300	1100	1145	1100	1000	930
8	K	4	Orange-lemon	32	0.73	1.45	1500	720	680	670	624	533	488	425
9	O	1	None	32	0.64	0.51	1107	1010	—	755	700	508	240	115
10	O	1	Lactic acid	32	0.64	1.97	1107	780	—	350	240	139	—	3
11	O	2	Lemon	32	0.90	1.95	2528	1810	—	900	—	440	48	26
12	O	2	None	32	0.90	0.75	2528	1670	—	1400	—	1050	347	160
13	O	3	Lemon-orange	32	0.57	1.95	1080	720	470	280	260	150	30	8.4
14	O	4	None	32	0.85	0.65	1300	900	1000	890	833	870	—	700
15	O	4	Lactic acid	32	0.85	1.52	1300	840	660	560	550	350	—	250
16	H	1	Lemon-orange	32	0.75	2.05	1550	540	460	—	—	450	240	—
17	H	1	None	32	0.75	0.63	1550	1000	730	—	—	820	555	—
18	H	1	None	20	0.75	0.63	1550	1250	750	—	—	790	600	—
19	A	1	None	32	0.48	0.40	412	340	—	300	—	98	53.5	—
20	A	1	Citric acid	32	0.48	1.08	412	360	—	210	—	27	5.0	—
21	A	1	Citric acid	32	0.48	1.37	412	320	—	180	—	25	5.0	—
22	A	2	None	32	1.10	0.78	850	620	600	—	210	180	—	160
23	A	2	Citric acid	32	1.10	2.10	850	680	490	—	180	160	—	20
24	A	2	Citric acid	32	1.10	3.40	850	560	512	—	42	35	—	3.5
25	A	3	Coffee	32	0.76	0.56	540	—	—	52	36	—	—	11
26	A	4	Coffee	32	0.83	0.61	410	—	—	36	27	—	7	—
27	A	5	Walnut	32	0.82	0.74	386	—	—	92	69	—	98	—
28	A	6	Grape	32	0.53	—	310	—	—	75	73	—	—	18

Where there are no data counts were not made.

maining after a storage period of seven days. Whether or not these variations were due to the age of the cells or some combination of factors was not determined.

Another point of interest was the apparent relation of reaction to the death rate of the organisms. Several sherbets carrying organisms from the same lot of cultured milk were prepared in such a manner as to differ in reaction only. This was accomplished by the addition of fresh lemon and orange juices, citric acid, or lactic acid to one of the duplicate mixes. Where this was accomplished by the use of lemon and orange juices, these juices constituted from 10 to 20 per cent of the volume of the mix. This dilution resulted in a natural decrease in organisms per unit volume as compared with the mix not receiving such an addition. Where concentrated solution of citric or lactic acid were used the reduction in numbers of organisms per unit volume due to dilution was a negligible factor. Sherbets 1 and 2 were prepared from the same sample of cultured milk, the acid content of sherbet 2 being increased from 0.47 per cent to 1.62 per cent by the addition of lactic acid. After a storage period of five days, the viable organisms in these sherbets numbered 700 million and 500 million per cc. respectively. Similar comparisons were made with sherbets 3 and 4, 9 and 10, 11 and 12, 14 and 15, 16 and 17, 19, 20 and 21, and 22, 23 and 24, and in every instance a greater number of viable organisms were found in the sherbet having the smaller amount of titratable acidity. These findings are similar to those reported by Black and Harris (5) in which they found the greatest destruction of organisms during storage at refrigerator temperature to occur in the most acid samples of the series.

In sherbets 5 and 6, and 17 and 18, a comparison was made of the effect of sugar on the viability of the organisms. Sherbets 5 and 6 were prepared with sugar contents of 35 per cent and 20 per cent respectively, and sherbets 17 and 18 were prepared with sugar contents of 32 per cent and 20 per cent respectively. After seven days storage, sherbets 5 and 6 contained 1,400 million and 1,600 million viable organisms per cc. respectively and after five days storage sherbets 17 and 18 contained 555 million and 600 million viable organisms per cc. respectively. Taking into consideration the variation in dilution caused by the addition of the respective amounts of sugar the difference in counts in the sherbets used in this comparison was so small as to be of no significance.

A number of very palatable sherbets were prepared in which grape juice, artificial walnut and coffee flavoring materials were used. These materials, as well as lemon and orange juices and crushed pineapple, served in a highly desirable manner to mask the natural *L. acidophilus* milk flavor or the cooked flavor imparted during the pasteurization of the milk.

Whether or not therapeutic benefits would result from the use of these sherbets prepared from *L. acidophilus* milk was not determined. According to Kopeloff (2) the average daily dose should be the equivalent of 1,000 cc. of a culture containing 200 million viable *L. acidophilus* organisms per cc. However, as reported by practicing physicians, the favorable results from the continued use of broth cultures prescribed in comparatively small daily doses along with lactose or mixtures of lactose and dextrose would tend to indicate that smaller doses are of benefit. It would seem that *L. acidophilus* sherbets of low acidity and carrying organisms of the more resistant strains if used within a period of seven days after manufacture, might possess therapeutic properties equal to if not better than the majority of broth cultures found on the market at the present time.

SUMMARY AND CONCLUSIONS

Twenty-eight sherbets were prepared from acidophilus milk and a study was made of the numbers of viable organisms remaining after 1, 2, 3, 5 and 7 days of storage at temperatures below -17°C. Four strains of *L. acidophilus* were used in the preparation of these sherbets.

A variation was found in the viability of these strains when exposed to the conditions prevailing in such a frozen product. The numbers of viable organisms in sherbets carrying strain A organisms were reduced to only a few million per cc. after five days storage, while under similar conditions several hundred millions of viable organisms were usually present in the sherbets carrying the other strains of *L. acidophilus.*

A variation in viability was found to occur in cultures of the same strain. Whether or not this variation was due to the age of the cells or some combination of factors was not determined.

A number of these sherbets were prepared so that they differed in reaction only. A more rapid reduction of organisms was found to occur in the sherbets of the higher titratable acidity.

The sugar concentration, as it was varied in the sherbet mixtures, caused no significant variation in the viability of the organisms.

It was possible to prepare sherbets in which large numbers of organisms remained viable after five to seven days of storage at temperatures below -17°C. when the more resistant strains of *L. acidophilus* with a high initial count were used and the titratable acidity was not permitted to become excessive, preferably not in excess of 1.0 per cent.

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A MICROSCOPIC STUDY OF ICE CREAM TEXTURE

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INTRODUCTION

The organoleptic test, although very valuable in certain respects, has not proved a satisfactory measuring device when applied to observations designed to evaluate factors influencing the texture of ice cream. Generally speaking, investigators have been slow to resort to a microscopic procedure which would determine the crystal structure of ice cream and, hence, its texture. It seems, however, that the earlier attempts to develop a microscopic technique which would supplement the organoleptic test should be regarded as significant.

Physical inconveniences associated with working at extremely low temperatures, coupled with the fact that the early attempts gave results which were far from satisfactory, possibly accounts largely for the fact that a microscopic method has not been more generally used. It is felt, however, that many erroneous conclusions drawn in the past, particularly those regarding the influence of freezing upon ice cream texture, would have been avoided had a more satisfactory method been employed as a basis of evaluating the factors under consideration.

HISTORICAL

Recognizing the disadvantages of the organoleptic test a few have resorted to various means of examining ice cream samples microscopically. A brief review of the reported attempts at a microscopic examination of ice cream texture follows.

Brainerd (1) made a study of the microscopic appearance of ice cream. Using indirect lighting, he examined ice cream frozen to a pipe refrigerated by the expansion of ammonia. The pictures included in his report showed a difference in crystal structure between coarse and smooth textured samples.

Hall (2) used a modification of Brainerd's technique and examined thin flakes or sheets taken from the surface of ice cream with the aid of dissecting needles. He used a relatively low magnification in his photomicrography.

Dahlberg (3) prepared free-hand sections of ice cream and by the use of light reflected from a mirror up through the sample, studied the struc-

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ture of crystals and their arrangement in ice cream. He tried hand microtome sections, but claimed better results from the free-hand sections.

Cole (4) was unable to get satisfactory free-hand sections, but by using a rotary microtome secured samples of ice cream which were satisfactorily examined under the microscope. The photomicrographs of these samples represented a wide range in the texture of ice cream. His pictures were taken with a Leitz "Macca" micro-camera and although various magnifications were used, a magnification of 100 × was most generally employed.

Munkwitz (5) prepared samples of ice cream for microscopic study in the following manner. Paper tubes about 3 inches in diameter and fifteen inches long were filled with ice cream and after hardening the samples, the paper tubes were cut and the columns of ice cream broken. The magnification used in this work was approximately 7 ×.

METHODS

The procedure included in this report is essentially the same as that developed in 1928 and briefly reported (6). It is given in more detail with certain modifications which are considered improvements over the procedure listed in the earlier reports.

Fixing the sample: The use of a fixative on the microtome sectioning attachments prior to freezing on the samples to be examined aided considerable in preventing them from becoming detached during the time of sectioning. Egg albumin or a dextrin medium[1] served the purpose adequately.

Sectioning: In addition to free-hand sectioning, various types of microtomes were tried, but the best results were obtained with a rotary type microtome. For the work reported here a Spencer rotary microtome (Cat. No. 820) was used. With this instrument it was possible to obtain ribbon sections four to six inches in length when conditions were properly controlled.

The temperature of sectioning had to be such that the samples did not stick to the knife which was the case when the temperatures were too high. It likewise could not be too low, otherwise shattering of the sample took place. Temperatures in the neighborhood of –18° C. were most commonly used. The correct bevel of the microtome knife was likewise important in determining the type of sections obtained.

Practically all of the sectioning was done with the microtome set to cut sections varying in thickness from 10 to 15 microns. Sections which were too thick could not be properly illuminated. If they were too thin they were difficult to handle and would usually tear.

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[1] The dextrin solution was prepared by adding dextrin to an aqueous solution of carbonic acid until a thick syrup was obtained.

Ice cream samples with very high overruns, also those having comparatively low freezing points, were the most difficult to section of any encountered in this study.

Microscopic observations: The microtome sections prepared as indicated above were carefully transferred to glass slides. Slight wrinkling of the samples was difficult to prevent, but it was possible to select portions of the sections which were satisfactory for microscopic examination. The use of a cover glass aided somewhat in this regard, since it tended to keep all parts of the section in the same plane. It was a decided advantage to have a flat section, although for microscopic observations slight irregularities in the sections did not materially interfere, since it was possible to vary the plane of focus during the observations. A magnification of approximately 100 × was most commonly used, but observations were made in which magnifications of 40 × and 430 × were employed.

With the aid of an ocular scale standardized against a stage micrometer scale, it was possible to measure the dimensions of air cells as well as of crystals being studied. This was done with many of the samples, but it was later felt that the reproduction of photomicrographs was much more satisfactory providing the latter were representative of the samples under observation.

Illumination: The method of illumination consisted of reflecting light from a mirror up through the section on the stage of the microscope. A Leitz radio lamp (Cat. No. 3865) was used with a daylight screen for the early observations. This was fairly satisfactory for microscopic observations and where Kodak film packs were used it gave satisfactory results with the work in photomicrography. When commercial panchromatic plates or films were used, it was found that the use of a yellowish-green filter and a Bausch and Lomb adjustable microscope lamp (6 volts) proved more satisfactory. The proper illumination is very essential for microscopic observation, but even more important as a means of obtaining satisfactory photomicrographs.

Photographic technique: A Bausch and Lomb type R camera proved satisfactory for this work. It was a big improvement over the small camera used earlier in the investigation. It is quite likely that several other cameras could be used with equal or possibly better satisfaction. Various kinds of films and plates were used, but it seemed that commercial panchromatic films or plates gave the desired contrast. As indicated elsewhere, special filters can be used to advantage, the most satisfactory type to use being dependent upon the light sensitivity of the films or plates. If dimensions of crystals or air cells are desired, measurements can be made on the photomicrographs themselves and then by taking into consideration the magnifications used the desired dimensions can be evaluated.

CONSIDERATION OF THE PHOTOMICROGRAPHS

In the accompanying photomicrographs, there is considerable variation in the size of not only the ice crystals but the air cells as well. Possibly a word of explanation by way of distinguishing between the air cells and the crystals is not amiss at this point.

Generally speaking, the air cells are somewhat whiter in appearance than the crystals or the associated substances and consequently in most cases can easily be distinguished on the pictures from them. The air cells do not always appear round which would be the case if they were spherical in shape. Instead their sides are irregular and hence outline rather fantastic figures in some cases. This tendency is more noticeable where the air cells are large, although not entirely absent in the case of small air cells as can be seen from the accompanying reproductions. There are probably several contributing factors but a very obvious one is the projection of irregular shaped ice crystals into these cells.

The ice crystals usually appear irregular in shape. They are not as light as the air cells and they do not appear as dark as the remaining constituents in most cases. Normally it would be expected that the directions of growth of the ice crystals would vary from crystal to crystal, such that the chances of two adjacent crystals growing in exactly the same plane and direction, would be extremely remote. The irregular shaped crystals represented on the pictures can be partly accounted for on this basis, since the sectioning which was done in one plane would result in the cutting of each of the crystals at a different angle. In addition, it is known that ice crystals themselves are not uniform in shape, which would also add to the chances for the irregularities noted.

It is felt that the pictures can be considered fairly representative cross-sections of the ice cream samples included in this report.

This study was undertaken in connection with a consideration of the influence of methods of freezing upon the texture of ice cream. Hence, many other factors which might have been included have not been given consideration here. Reference to a previous article will give the details of this part of the study (7). It is felt, however, that so far as composition is concerned the ice creams studied are fairly representative of commercial products.

Plate 1, no. 1, represents an ice cream frozen by simply placing a pint of mix in the hardening room held at about –18° C. with no agitation during the freezing process. The sample was very coarse, which is indicated by the large ice crystals as well as the tendency for them to be arranged in strata. It was easily identified as an icy sample by the organoleptic test.

The samples represented by plate 1, no. 2, and plate 2, nos. 1 and 2, were all made from the same mix, but were drawn from the freezer at –2.7°

C. (27.1° F.), –3.3° C. (26.1° F.) and –4.4° C. (24.1° F.) respectively. The freezing process was completed by placing a quart of each of the samples in the hardening room along with the first sample mentioned. There was a significant difference in the crystal structure of these samples, increasing in smoothness as the temperature of drawing from the freezer was lowered.

The composition of the samples represented by plate 3, nos. 1 and 2, was essentially the same as the three previous samples. Plate 3, no. 1, was drawn from the freezer at –6.1° C. (21.0° F.) and a quart subsequently placed in the hardening room along with the other samples. The crystal structure indicated by the photomicrograph shows it to be much smoother than any of the samples so far considered. The sample represented by plate 3, no. 2, was frozen by placing a small quantity of the mix on a carbon dioxide freezing attachment. It was frozen solid in less than three minutes and as can be seen was much smoother than any of the other samples.

The contrast between the texture represented by the two photomicrographs of plate 4 indicates the changes which can occur in the texture of samples of ice cream where extreme fluctuating temperatures occur during storage. Plate 4, no. 1, shows the texture of the ice cream the day after it was drawn from the freezer while no. 2 shows the same sample 5 days later. This change occurred as a result of fluctuations in temperature during that time.

Plate 5 represents two commercial samples of ice cream which are probably better so far as texture is concerned than average commercial ice cream.

DISCUSSION

The introduction of a microscopic technique for measuring ice cream texture should be regarded as significant. Although the early attempts were accompanied by considerable handicaps, yielding results which were not very satisfactory, yet the development of the method seems to make these measurements more quantitative than was possible where the organoleptic test was the only recourse for investigators in this field.

Inadequate illumination as well as improper sectioning seem to be the most common sources of trouble where this method has been employed. Even though satisfactory photomicrographs are not always obtained and possibly are not necessary for certain types of work, it seems that the microscopic examination of prepared sections might find more general application. It is likely that its best use will be in scientific observations, although it may find some commercial applications as well.

It is recognized that by means of the tongue it is possible to determine relative differences in sizes of particles which might be taken in the mouth.

The degree of accuracy with which this can be determined is, however, a variable dependent, among other things, upon the individual making the determinations as well as upon the range in size of the particles being examined. On the other hand, the range over which the size of particles can be accurately determined microscopically is infinitely greater and hence should find greater application than the former method. In this connection it is of interest to note that the sample represented by plate 4, no. 2, was judged to be smooth organoleptically, even after the extreme fluctuations in temperature noted. It was judged to be of approximately the same smoothness as the sample represented by plate 5, no. 1. A comparison of the two photomicrographs mentioned will show them to have approximately the same texture. It can be seen further that their texture is relatively coarse compared with plate 3, nos. 1 and 2.

The coarse texture represented in plate 1, no. 1, may possibly be partly accounted for by the composition of the mix which was relatively low in fat. When that same mix was frozen rapidly its texture approached that represented by plate 3, no. 2, which compared favorably to a fine fondant in smoothness. This indicates that the major factor here was rate of freezing rather than composition.

Plate 4, no. 1, shows a smooth textured product which incidentally compares favorably with plate 3, no. 1, the two samples being frozen under almost the same conditions. It is much smoother, however, than plate 4, no. 2, which was the same sample of ice cream subsequently stored under very unfavorable conditions. The treatment was more severe than is commonly the case in commercial operation; on the other hand, the sample, to begin with, was much smoother in texture than most commercial ice creams.

Fluctuating temperatures are conducive towards the growth of large crystals at the expense of smaller ones. This may be due to the fact that for a given mass the smaller the crystals the greater their surface area and hence the greater their tendency to dissolve or go into solution. On this basis, as the temperature was increased the smaller crystals would melt first, some of them disappearing entirely. Then when the temperature was subsequently lowered, the water which had been formed as a result of the melting, would freeze around the nuclei of the crystals which were relatively large to begin with, thus causing them to increase in size with each cycle of the fluctuations in temperature.

In addition the radius of curvature of the smaller particles and hence the escaping tendency of the molecules from their surface would exceed this tendency were the radius of curvature greater. As a result of this phenomenon the tendency would also be for the large particles to grow in size at the expense of the smaller ones of the same composition. These two tendencies are in agreement with the experimental facts and it is logical to conclude that they are contributing factors, although it is fairly certain that there are other factors concerned with these changes.

This study substantiated the conclusion already drawn (7), *viz.*, that the rate of freezing materially affects the size of the crystals formed in ice cream and further that proper methods of freezing and storage can be expected to correct many defects common to ice cream texture. It was not possible, however, to correlate the smoothness of texture as determined by the size of the ice crystals with the size or volume of air cells in the ice cream.

ACKNOWLEDGMENT

The author gladly acknowledges the assistance of Mr. Herbert Sprague, who spent considerable time working at disagreeably low temperatures assisting with the photographic work included in this report.

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EXPLANATION OF PLATES

PLATE 1

No. 1. Mix frozen by placing a pint of it in the hardening room maintained at about –18° C. (0° F. approx.). Composition: 6% fat, 15% sugar, 0.35% gelatin and 32.35% total solids. Freezing point –2.50° C. Magnification 100 ×.

No. 2. Mix drawn from a 40-quart direct expansion freezer at –2.7° C. (27.1° F.) and the freezing completed by storing sample in hardening room at about –18° C. (0° F. approx.). Composition: 12.0% fat, 15.0% sugar, 0.45% gelatin and 38.6% total solids. Freezing point –2.45° C. (27.6° F.). Magnification 100 ×.

PLATE 2

No. 1. Sample from same run as plate 1, no. 2, drawn from the freezer at –3.3° C. (26.1° F.) and the freezing completed as before. Magnification 100 ×.

No. 2. Sample from same run as plate 2, no. 1, drawn from the freezer at –4.4° C. (24.1° F.) and the freezing completed as above. Magnification 100 ×.

PLATE 3

No. 1. Sample drawn from a 40-quart direct expansion freezer at –6.1° C. (21.0° F.) and the freezing completed by placing it in the hardening room as previously described. Composition: 12% fat, 15% sugar, 0.35% gelatin and 37.85% total solids. Freezing point –2.69° C. (27.2° F.). Magnification 100 ×.

No. 2. Same mix as in plate 3, no. 1, but frozen solid in less than three minutes by placing a small amount of it on a carbon dioxide freezing attachment. It was subsequently placed in the hardening room with the other samples. Magnification 100 ×.

PLATE 4

No. 1. The mix from which this sample was made, although not processed at the same time, was standardized to have the same composition as the mix used in plate 3, no. 1. The sample was drawn from a 40-quart direct expansion freezer at a temperature of –6.0° C. (21.2° F.) and the freezing completed as with the other samples. Magnification 100 ×.

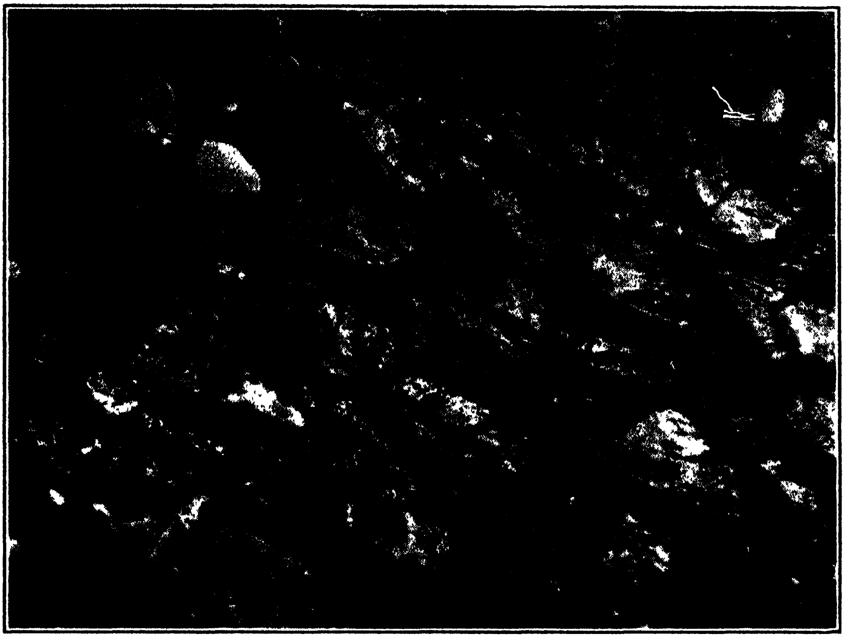
No. 2. This represents the texture of the same ice cream as in plate 4, no. 1, after the sample had been stored for 5 days, during which time wide fluctuations occurred. This was accomplished by removing the quart sample from the hardening room maintained at about –18° C. and placing it in a room at about 0° C. until the middle had reached a temperature of approximately –8° C. It was then returned to the hardening room. This fluctuation in temperature occurred 6 times in the 5-day period after hardening and before sectioning. Magnification 100 ×.

PLATE 5

No. 1. This represents the texture of a sample of commercial ice cream entered in the ice cream scoring contest at the Pacific Slope Dairy Show, November, 1931. The judges gave it a perfect score on body and texture, but at the time of photographing it was not quite as smooth as it was when scored officially. Analysis of the sample showed it to contain 13.79% fat, 40.31% total solids. Magnification 100 ×.

No. 2. This represents another commercial sample entered in the same contest mentioned above. It was criticized as coarse and scored 24.0 points for body and texture. This sample had likewise increased in coarseness to some extent before it was sectioned and photographed. It was, however, stored under the same conditions as the sample represented by plate 4, no. 1, after entering the contest. Analysis of the sample showed it to contain 13.72% fat, 37.09% total solids. Magnification 100 ×.

PLATE 1



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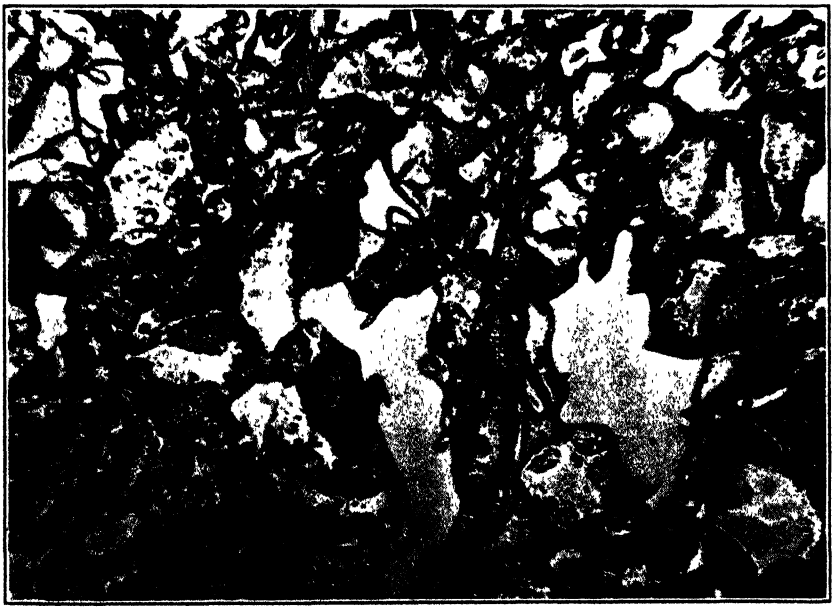

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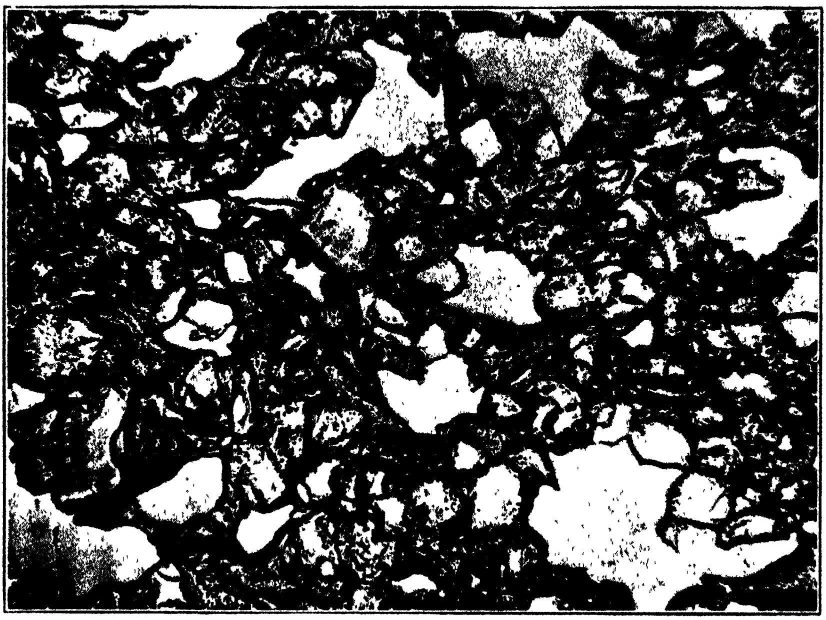
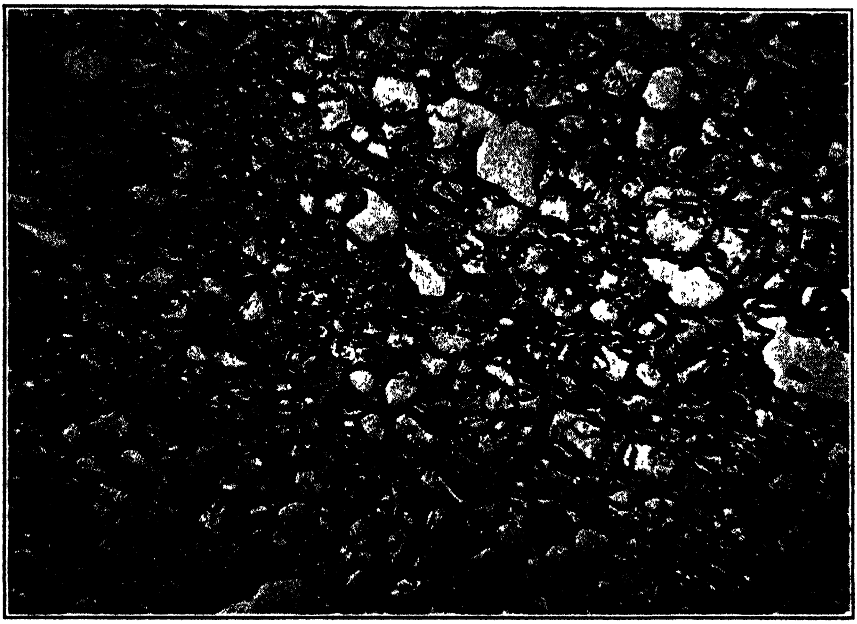
PLATE 2



No. 1

No. 2

PLATE 3



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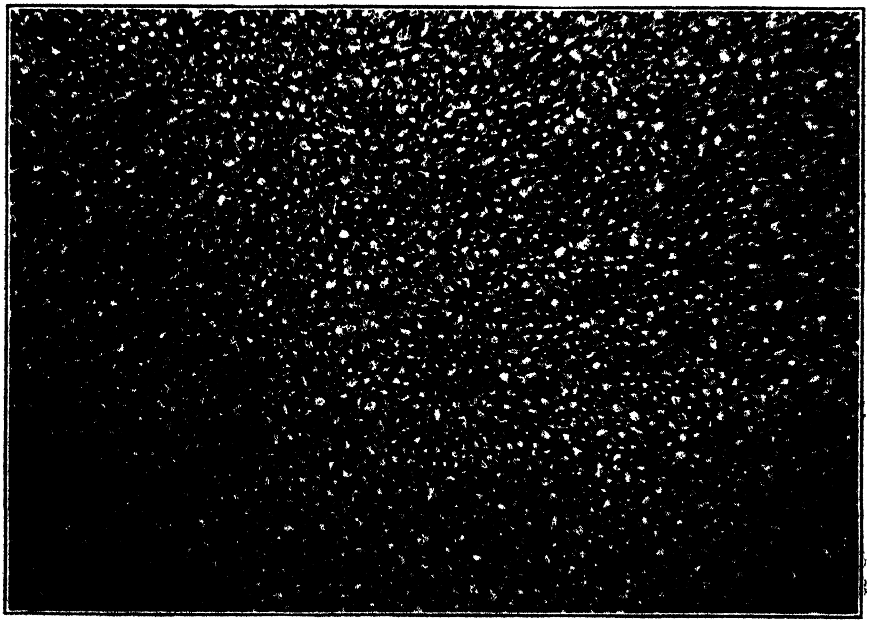

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PLATE 4

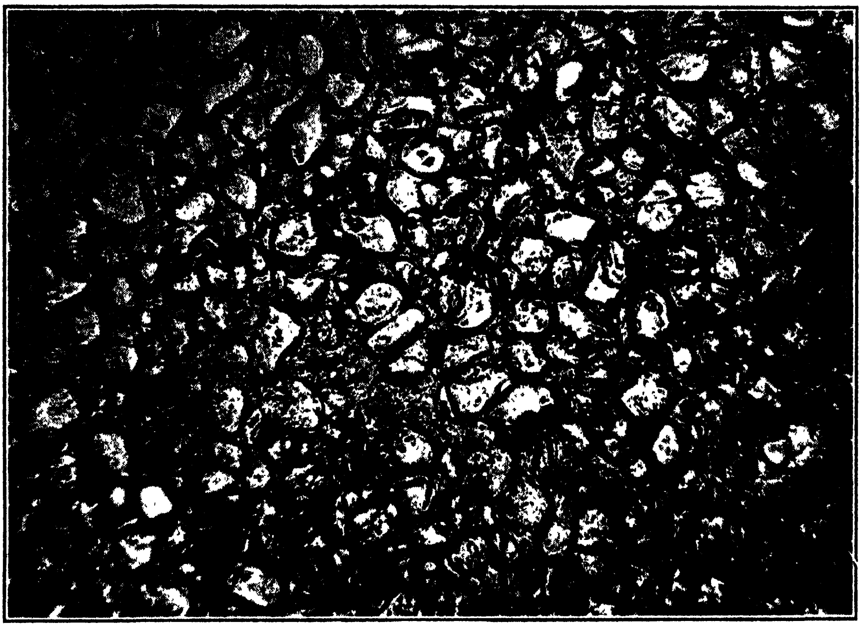

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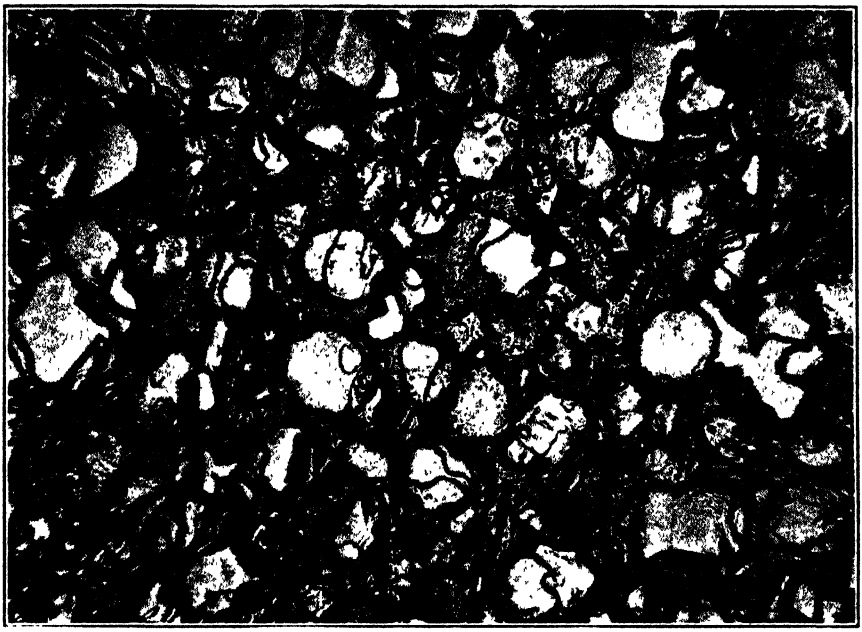
PLATE 5



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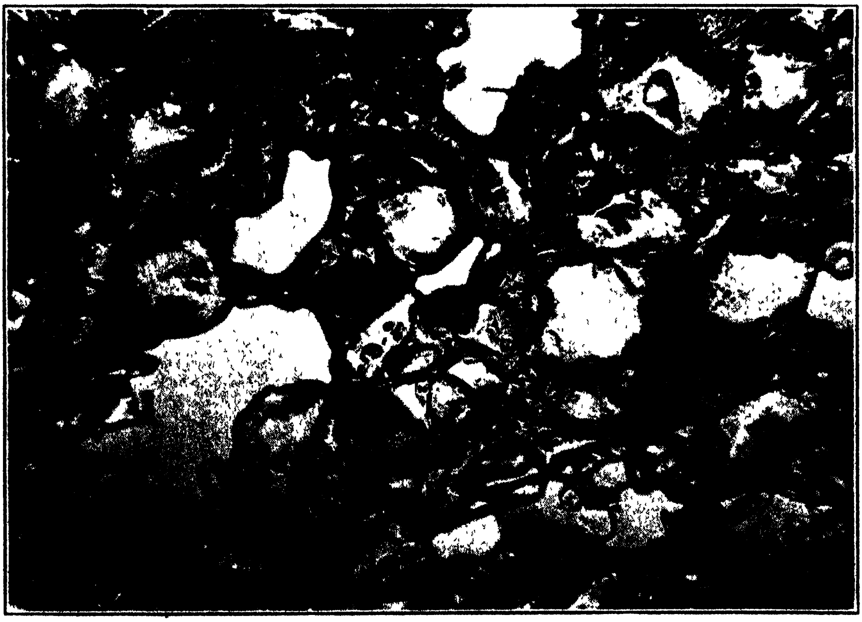

No. 2

A STUDY OF THE EFFECTS OF ALFALFA HAY AND IRRIGATED PASTURE ON GROWTH OF HOLSTEIN HEIFERS*

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The results of these investigations indicate to what extent large, growthy heifers may be developed on the common feeds produced in Wyoming. Alfalfa hay was selected as the roughage because it is found on most every irrigated farm in Wyoming where dairy cattle are kept. The number of acres in the state seeded to alfalfa is slightly below that of wild hay, but the yield is twice as great as that of wild hay, making the total tonnage of alfalfa produced much greater.

Barley was selected as the concentrate for one group of heifers, the object being to determine if a small allowance of grain would aid in producing better developed dairy cattle. The acreage of barley grown in the state increased 500 per cent from 1920 to 1928. In 1929 the acreage of barley was below the acreage of corn. Because of the greater yield of barley per acre the total production in the state was 50 per cent more than that of corn. Barley production in Wyoming has several advantages over corn. Less than one per cent of the area of the state is below 4,000 feet in altitude, and 95 per cent of the area is above 5,000 feet in altitude. Barley thrives in high altitudes where the growing season is too short and the nights too cold for corn to mature.

Two types of irrigated pastures are in use on farms in Wyoming—pastures composed of perennial grasses and pastures composed of sweet clover. Both types of pastures were grazed by heifers in this experiment.

REVIEW OF LITERATURE

It has been shown by Eckles (5, 6) that age at first calving influenced the size of cows at maturity; that a Holstein heifer should be bred to drop her first calf when 28 to 30 months old if maximum development is desired. Eckles (8) compiled growth records of a number of heifers raised at the University of Missouri. From these data Eckles drew a curve of normal growth from birth to maturity, as represented by weight and height at withers. This curve has been found useful as a basis for comparison for growth of experimental animals. In the report on normal growth, Eckles made note of the fact that "if an animal was above or below normal at a

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certain age it tends to remain about the same distance above or below normal for a long period.'' Using data collected from the University of Missouri dairy herd, Brody and Ragsdale (2, 3) report changes in body measurements during growth of dairy cattle and also a height-weight curve (1).

The influence of the ration on growth of dairy heifers has been shown by a number of investigations. Eckles (5, 6) showed that the difference between heavily and lightly fed heifers was more marked upon the weight than upon the skeletal growth. The ration of Eckles light fed group after six months of age consisted of alfalfa hay alone. The ration of light fed group in experiments reported by Reed (11) was similar to that of Eckles data. The light fed heifers in both experiments failed to reach ''normal'' size. The Nebraska Agricultural Experiment Station (4) showed a wide difference in development of heifers on light rations and heavy rations. Woll (13) fed heifers on alfalfa hay alone in winter and green alfalfa in summer. The average weight of the Holstein heifers at the start was 687 pounds at an average age of 13 months. These heifers made an average daily gain of 1.05 pounds up to freshening—a gain somewhat above normal rate. At the Huntley Montana Experiment Station (10) Holstein heifers were fed skim milk up to 9 months of age, then only alfalfa and corn silage with two months pasture during their second year. At 12 months of age these heifers averaged 721.2 pounds in weight as compared to Eckles normal weight for that age of 558 pounds. From 12 to 24 months of age the Huntley heifers gained at more than normal rate. The Huntley heifers were larger at 24 months of age than those raised at Beltsville and Ardmore Station, where a small grain allowance was given.

The most extensive work on rations for wintering dairy heifers was done by Eckles (7). In this work the wintering of 76 animals of various ages on different rations was reported. On the average the heifers wintered on alfalfa hay alone failed to make normal gains. Of the seven Holsteins in Group 2, Experiment 1, only one, a 14 months old heifer, exceeded normal gains. All of the Holstein heifers were below normal size at the beginning of the experiment and varied in age from 6 to 15 months. Heifers receiving 2 pounds of corn daily in addition to alfalfa hay grew at normal rate at all ages. Eckles found that summer gains were in the reverse order to winter gains, but where gains were normal during the winter, normal pasture gains would continue. He also concluded that Holsteins were better able to thrive on roughage alone than Jerseys. The South Carolina Station (12) secured normal growth feeding heifers for three months on alfalfa hay alone. The Idaho Station (9) failed to secure normal growth when wintering heifers on alfalfa hay alone.

From the above investigations it would seem that Holstein heifers might grow at normal rate and reach a satisfactory size on roughage alone, beginning at 12 to 13 months of age, if the heifers were of more than normal size.

EXPERIMENTAL PROCEDURE

In the research reported here, twenty registered Holstein heifers, varying in age from six months to twenty-four months, were divided into two lots. During the winter the two lots of heifers were fed in separate corrals 25 x 125 feet in size. Each lot had access to a shed open on the south side, 18 x 25 feet in size.

During the winter the heifers were weighed for three consecutive days every thirty days. On the middle day of each weighing period the following measurements were taken: height at withers, width at hooks, depth of chest, circumference of heart girth, and circumference of middle. During the summer, weights were taken once every thirty days. At this time the regular measurements were also made. The heifers were bred to calf at about thirty months of age.

RATIONS FED

During the winter, Lot I received alfalfa hay exclusively, while Lot II received alfalfa hay and two pounds of ground barley per head daily. During the summer, both lots were placed on irrigated sweet clover and tame grass pasture with no grain supplement for either lot. During the winter months, the heifers were fed alfalfa hay. The hay was weighed out daily into a hay bunk. The heifers were allowed hay in slight excess of that which they would clean up. From time to time the bunks were cleaned out of all refused hay and the weigh back recorded. Second cutting alfalfa was fed, and for the most part would grade as U. S. No. 2. Block salt and water were available at all times. Ground barley was fed in a feed bunk, to which all heifers in Lot II had access.

TABLE 1

Average weights and gain in weight of heifers at 12, 16 and 27 months of age in Lots I and II

AGE IN MONTHS	AVERAGE WEIGHT IN POUNDS			POUNDS GAIN IN WEIGHT		
	GROUP 1	GROUP 2	AVERAGE	GROUP 1	GROUP 2	AVERAGE
			*Lot I**			
12	660		660			
16	764	769	766	104		104
27	1140	1128	1136	376	359	370
			*Lot II***			
12	605		605			
16	743	860	760	138		138
27	1110	1183	1128	367	323	368

*Group 1 contained 5 heifers, group 2 contained 4.
** Group 1 contained 6 heifers, group 2 contained 2.

PRESENTATION OF EXPERIMENTAL DATA

The heifers in each lot may be divided into two groups: those placed on experimental rations at 12 and 13 months of age (group 1, table 1) and those placed on experimental rations at 16 and 17 months of age (group 2, table 1). In Lot II there were three heifers which were placed on experimental feeding at the age of 6 to 8 months. One of these heifers, however, was taken off the experimental rations at 14 months of age and her monthly weights are not included for the present. The monthly weights of the other two heifers are only considered beginning at 12 months of age. Table 1 shows the average weights of the groups in both lots at 12, 16 and 27 months of age.

As may be seen in table 1, the heifers in Lot I beginning the experiment at 12 months of age were heavier at the start and at 27 months of age than the heifers in Lot II. When all heifers are taken into consideration those in Lot I were slightly heavier than those in Lot II at 27 months of age. The gains made during specified periods are also shown in Table 1.

As shown in table 1 the heifers in Lot I did not make such rapid gains from 12–16 months of age as the heifers in Lot II. In group 2 the heifers in Lot I grew at a more rapid rate than the heifers in Lot II. When all heifers are considered, the heifers in Lot I grew as rapidly as the heifers

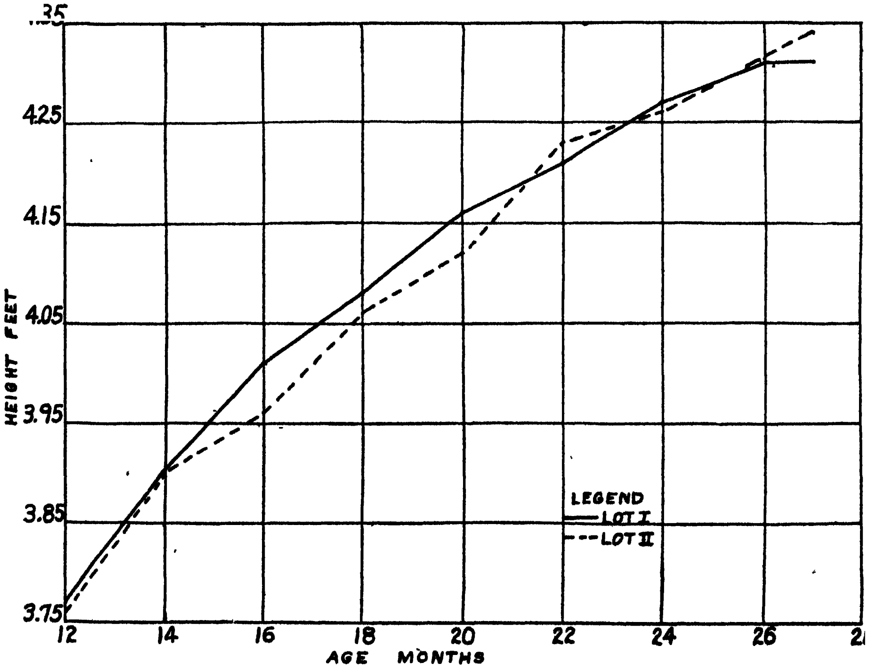

FIG. 1. SHOWING RATE OF GROWTH AS MEASURED BY HEIGHT AT WITHERS OF ALL HEIFERS IN LOTS I AND II.

in Lot II after the heifers in Lot I had reached the age of 16 months. Considering the gains of all heifers from 12 to 27 months of age, those in Lot II, or the grain fed lot, grew at a more rapid rate than the heifers in Lot I which did not receive grain.

Figure 1 shows graphically the rate of growth as measured by height at withers of all heifers. The heifers in Lot I were taller at 12 months of age than the heifers in Lot II. At 27 months of age the heifers in Lot II were slightly taller than the heifers in Lot I. The difference, however, might be said to be insignificant.

WINTER GAINS

During the winter feeding, the heifers were of varying ages. Since the hay allowance was offered to the group, the hay consumption of individual animals was not secured. The winter gains made by heifers in Lots I and II grouped by ages at start of winter feeding period is shown in table 2.

TABLE 2

Average monthly gains made during the winter by heifers in Lots I and II grouped by ages at start of winter feeding

LOT NO.	YOUNGER HEIFERS			OLDER HEIFERS		
	NUMBER HEAD	AGE AT START	AVERAGE MONTHLY GAIN	NUMBER HEAD	AGE AT START	AVERAGE MONTHLY GAIN
		months	*pounds*		*months*	*pounds*
I	4	12.25	22.40	8	19.63	31.39
II	5	9.20	31.30	6	17.33	32.35

The average length of time on winter feed extended over a period of 7.5 months. Table 2 shows that the heifers which started at 12 months of age on alfalfa alone (Lot I) did not gain as rapidly as those on alfalfa alone at an older age. It was noted in the case of two heifers which were fed for two winters that their gains made during the second winter were larger than during the first winter. Table 2 also shows that the heifers of Lot II starting at 9 months of age made larger gains than the heifers of Lot I starting at an average age of 12 months. In addition to this, note should also be taken of the fact that the heifers of Lot II starting at an older age did not make much larger gains than the heifers in the same lot starting at a younger age.

The average monthly gain of both the younger and older heifers in Lot I was 28.5 pounds as compared with 32.0 pounds for Lot II. The average monthly gain of Lot II over Lot I of 3.5 pounds is not statistically significant.

PASTURE GAINS

No grain was fed to either lot on pasture. The heifers were run on pasture continuously for a period of 3.5 months. Lot I made an average monthly gain of 35.14 pounds as compared with 44.67 pounds made by Lot II. The heifers on both lots made larger monthly gains on pastures than on their respective rations during the winter months. The average monthly gain for all heifers during the summer on pasture was 40.6 pounds as compared with 30.3 pounds while on winter feed.

COMPARISON WITH DATA FROM OTHER EXPERIMENT STATIONS

The average weights of Holstein heifers in Lot I at 12, 24, and 27 months of age are compared with the weights of heifers grown under different feeding conditions at other Experiment Stations in table 3.

TABLE 3

*Average weights of Holstein heifers in Lot I at 12, 24, and 27 months of age compared with average weights of Holstein heifers at the Huntley, Montana Station; Missouri heavily fed late calving group; Eckles normal; and Missouri lightly fed late calving group**

AGE IN MONTHS	WEIGHT IN POUNDS		
	12	24	27
Lot I, Wyoming	660.0	1043.0	1138.0
Huntley, Montana	721.2	1070.8	..
Missouri Heavily Fed	664.0	1110.0	
Eckles Normal	558.0	841.0	925.0
Missouri Lightly Fed	432.0	731.0	.

* Method of feeding cited in review of literature.

At 12 months of age the Wyoming heifers showed as great a scale as the Missouri heavy feds, but not the scale of the Huntley heifers. All three groups mentioned were above Eckles normal at twelve months and 24 months of age. The Missouri light fed heifers on the other hand were below normal at 12 and 24 months of age.

The growth as measured by height at withers of Lot I is compared in figure 2 with Holstein heifers grown under different feeding conditions at other Experiment Stations. Figure 2 shows that the Wyoming heifers were above normal at 12 months of age and grew at more than normal rate. The Kansas and Missouri lightly fed heifers show retarded development. The Wyoming heifers were below the Missouri heavily fed heifers at 12 months of age but were slightly above at the age of 24 months.

Other measurements taken besides height at withers show that the Wyoming heifers were above normal at 12 and 27 months of age. The Wyoming heifers gained for the most part at normal or above normal rate as shown in table 4.

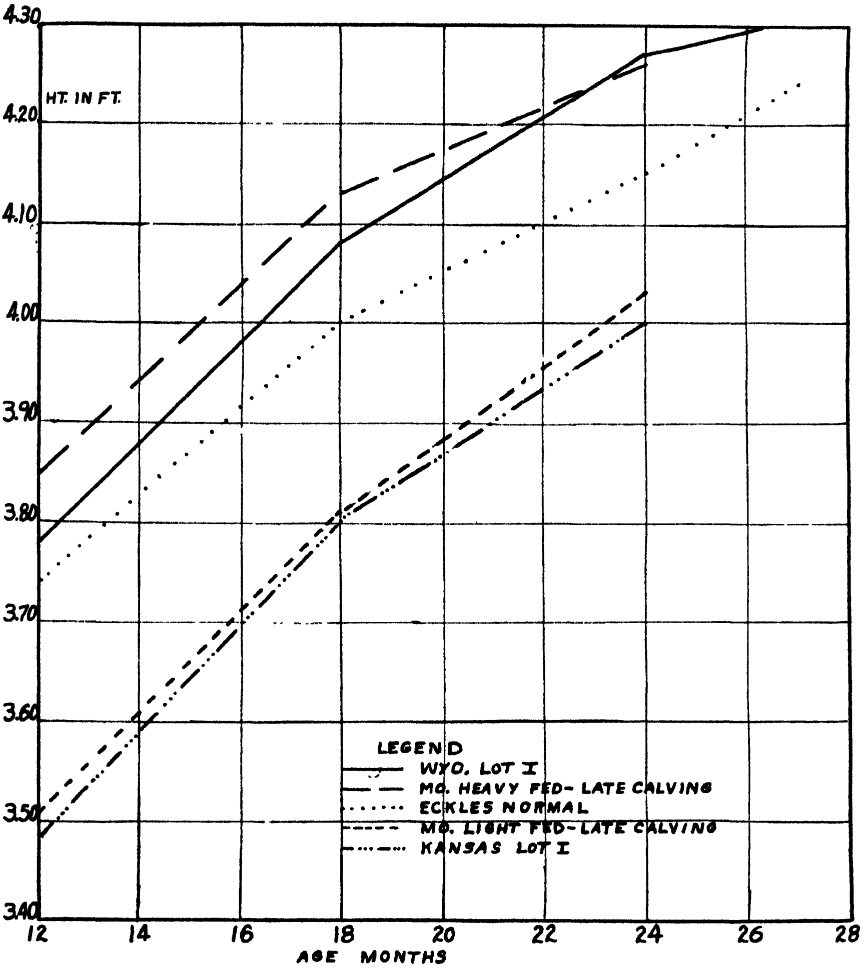

FIG. 2. SHOWING GROWTH AS MEASURED BY HEIGHT AT WITHERS OF HOLSTEIN HEIFERS FED AT MISSOURI, KANSAS AND WYOMING EXPERIMENT STATIONS.

TABLE 4

Average gain in weight and body measurements of all Wyoming heifers compared with normal between ages of 12 and 27 months

	WEIGHT	HEIGHT AT WITHERS	DEPTH CHEST	HEART GIRTH	BARREL GIRTH	WIDTH HIPS
	pounds	*feet*	*feet*	*feet*	*feet*	*feet*
Wyoming Lot I	478	.53	.37	1.10	1.27	.33
Wyoming Lot II	553	.57	.46	1.28	1.59	.44
Normal	367	.50	.37	.98	1.19	.37

The fact that the Wyoming heifers were in a good state of flesh is shown in table 5 in which the corresponding weight for height is compared with normal. A height-weight growth curve based on data secured from the Wyoming heifers would show that the Wyoming heifers were heavier at a given height than the Missouri heifers.

TABLE 5

Corresponding weight for height of the Wyoming heifers compared with normal

HEIGHT IN FEET	NORMAL WEIGHT	WEIGHT LOT I	WEIGHT LOT II
3.76	570	660	599
4.00	686	766	807
4.26	966	1043	1047

WINTER AND SUMMER GAINS COMPARED TO NORMAL

Since the heifers in this experiment were of different ages during the winter and summer, the growth of each heifer during those periods was compared with Eckles normal. Table 6 shows how the average rate of growth during the winter and pasture season compared with the normal rate.

TABLE 6

Average variation from normal weight and height of heifers in Lots I and II while on winter feed and pasture

SEASON	NO. HEAD	VARIATION FROM NORMAL WEIGHT IN POUNDS			VARIATION FROM NORMAL HEIGHT AT WITHERS IN FEET		
		AT START	AT END	GAIN OVER NORMAL RATE	AT START	AT END	GAIN OR LOSS ON NORMAL RATE
Winter							
Lot I	11	157.0	175.7	18.7	.080	.080	0
Lot II	11	107.8	154.0	46.2	.008	.087	–.079
Pasture							
Lot I	6	158.0	182.1	24.1	.093	.078	–.015
Lot II	11	120.3	172.7	52.4	.069	.060	–.009

As shown in table 6 the average gains in weight on winter and summer feed were above normal in both Lots I and II. During the winter Lot I made normal gains in height at withers while Lot II exceeded normal rate in the same measurement. Both lots failed to make normal gains in height at withers while on pasture. In weight the heifers in Lot II exceeded normal gains more than the heifers in Lot I both on winter feed and on pasture. While on pasture the gain in weight over the normal rate exceeded the gain during the winter in both lots.

There was considerable variation in the gains made by individuals in each lot. In Lot I three out of eleven head and in Lot II four out of eleven head failed to make normal gains in weight during the winter. At the same time five out of the eleven head in Lot I and three out of the eleven head in Lot II failed to make normal gains in height at withers.

While on pasture one heifer in Lot I and two heifers in Lot II failed to make normal gains in weight. The fact that heifers receiving grain during the winter (Lot II) made the larger gains during the summer seems contradictory to usual results. Eckles (7) found, however, that heifers medium fed during the winter showed no such increase in weight during the summer as did the light fed heifers. The winter gains on alfalfa alone might be considered satisfactory.

DISCUSSION

As shown by increased rate of gain of Lot II over Lot I, the addition of two pounds of barley to the alfalfa ration during the winter months was beneficial. The heifers in Lot I were larger than the heifers in Lot II at 12 months but not at 27 months of age. From the standpoint of the practical farmer, however, the differences in the two groups are insignificant. The final weights and measurments of the two groups are very close. The work reported by Woll (13) indicates that grain feeding increased the rate of growth of heifers. However, the heifers fed alfalfa alone might be said to have made satisfactory gains.

The point of view as far as relative growth is concerned depends upon whether one is to consider normal gains as satisfactory gains. The writer believes that normal gains should be considered sufficient as long as heifers are large for their age at the time of their first calf. From this standpoint it would appear that there is no special benefit derived by feeding grain to heifers when raised under the condition of this experiment. The experiment does not show at what age exclusive hay feeding may be safely begun, but it demonstrates that large twelve months old heifers may be raised satisfactorily on alfalfa hay and irrigated pasture.

SUMMARY AND CONCLUSION

Two groups of Holstein heifers were fed on alfalfa hay as a sole roughage during the winter and grazed on irrigated pasture during the summer. One group (Lot II) received two pounds of ground barley per head daily during the winter months, while the other group (Lot I) received no grain supplement.

The data indicate that size of heifer may still be a factor influencing rapidity of growth on roughage alone even up to the age of 16 months. During the winter months the grain fed heifers made an average monthly gain of 32 pounds as compared with an average monthly gain of 28.5 pounds for the heifers fed exclusively on alfalfa hay. The older heifers made larger gains than the younger ones on alfalfa alone.

The average monthly gains on pasture for all heifers in both lots was 40.58 pounds, as compared with 30.33 pounds on winter feed. The heifers receiving ground barley during the winter made larger gains on pasture than the heifers fed alfalfa hay exclusively. Little significance can be attached to this fact, however, in view of the limited numbers involved and also because the heifers fed alfalfa hay exclusively made quite satisfactory gains during the winter months.

In comparison with growth data from other Experiment Stations, the Wyoming heifers in Lot I made a favorable showing. The Wyoming heifers made more than normal gains in body measurements as well as live weight. The Wyoming heifers were heavy for their height as compared with normal.

Even though the grain fed heifers grew at a more rapid rate, the two groups were very much the same size at 27 months. For that reason the growth of the heifers in Lot I may be considered satisfactory.

In this investigation there seems to be two reasons for the successful growth of heifers without grain:

1. The heifers were of sufficient size at twelve months of age to consume enough roughage for desirable growth.
2. The possible beneficial effect of the irrigated pasture.

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THE VITAMIN "B" AND "G" CONTENT OF DRY SKIMMED MILK AND DRY WHEY

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Since the multiple character of the water soluble vitamins has been more clearly recognized, milk has been subjected to different empirical methods of assay for the purpose of determining its relative value as a source of vitamins "B" and "G."[1] The results of these studies (1, 2, 3, 4, 5, 6, 7) are confirmatory in showing that the vitamin "B" content of milk is relatively less than its vitamin "G" content. While the methods used for obtaining these results have not been uniform, nevertheless, the data clearly show that, aside from specific manifestations of polyneuritis which may occur in the test animals under suitable conditions of experimentation, there is also a retardation of the growth rate which can be attributed specifically to inadequacy of vitamin "B."

For the purpose of estimating the relative amount of vitamin "B" and "G" in various milk products, a method previously reported from this laboratory (6) has been found to be of value. The method is based on numerous data which have shown that with a basal ration containing a meticulously purified casein, supplemented with 2 per cent rice polish, white rats obtained a sufficiency of vitamin "B" to prevent polyneuritis and at the same time maintain a constant weight for several weeks due to the small amount of vitamin "G" carried by the rice polish. The test substance when superimposed on such a ration and when fed in graduated amounts causes a growth response commensurate with the amount of vitamin "G" present. Parallel feeding of the test substance superimposed on the basal ration unsupplemented with rice polish provided a series of graduated growth rates, which in conjunction with data obtained from the ration supplemented with the rice polish can be interpreted as a measure of the relative amount of vitamin "B" in the product being tested.

EXPERIMENTAL

The above method has been used for determining the relative vitamin "B" and "G" content of certain desiccated milk products, the results from

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[1] Vitamin "B" and vitamin "G" as referred to herein will be used in conformity with the recommendations of the Committee on Nomenclature of vitamin "B" of The American Society of Biological Chemists. Science 69: 276. 1929.

which will be reported herein. Since it is generally known that high temperatures have a destructive effect upon vitamin "B," and since there is some evidence (8, 9, 10) that such an effect may result from the degree of heat used in the processing of certain milk products, dry skim milk desiccated by the Just roller process and three samples of desiccated whey were used in this study. The temperatures to which the dry skimmed milk was subjected varied between 240° F. and 258° F. during a period which did not exceed 2½ seconds (11). The dry whey designated as sample "K" was sweet rennet whey suitable for human consumption, which had been desiccated by the Krause spray process. Dry whey samples "Ko" and "P" were obtained on the commercial market and definite knowledge of the methods of desiccation and temperatures applied are not known.

White rats selected from our standardized stock colony, 22 to 26 days of age and weighing from 40 to 50 grams, were used. They were kept in individual metal cages with raised screen bottoms during the test period. The basal ration was the same as used for previous studies (6) of this character and consisted of dextrin, 63 parts; Casein, 20 parts; powdered agar agar, 2 parts; Crisco, 10 parts; salt mixture No. 40,[2] 4 parts; and cod-liver oil, 1 part. For the required parallel feeding, this ration was used with and without the 2 per cent rice polish supplement. Varying percentages of the test products were fed with each of these rations. Since dry skimmed milk contains approximately 35 per cent protein and the dry wheys from 14 to 17 per cent, the protein content of the experimental rations was adjusted by removing part of the casein of the basal ration at the higher levels at which the test substances were fed. This procedure was followed in order to avoid a disproportionate protein content at different levels.

The growth curves are shown in the accompanying charts. Each curve represents the average growth rate of 4 rats, 2 males and 2 females, with one or two exceptions. The curves show that in every instance where the test product was supplemented with rice polish an increased rate of growth occurred. This is obviously the result of the supplementing effect of the rice polish and the milk products. The lower growth rate obtained when the basal ration was unsupplemented with rice polish must be attributed to the fact that the test products do not carry a sufficient amount of both vitamins "B" and "G" to give the same growth response at comparable levels as was obtained when the limited amount of rice polish was furnished. Since it is known that rice polish has a high vitamin "B" content and relatively little vitamin "G," at least sufficient to promote growth at the level furnished in these rations, the growth results from the unsupplemented milk products interpreted in conjunction with those obtained when

[2] Steenbock, H., and Nelson, E. M., Jour. Biol. Chem. 56: 362. 1923.

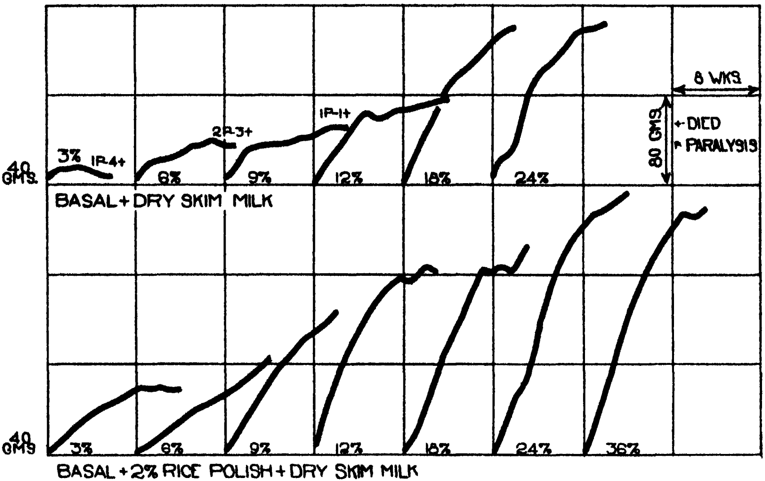

CHART 1. COMPARATIVE GROWTH RESULTS OF A VITAMIN B AND G ASSAY OF DRY SKIM MILK.

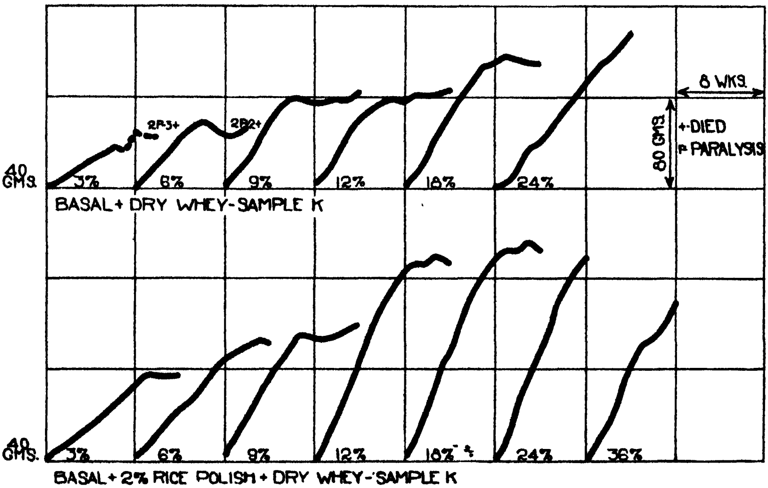

CHART 2. COMPARATIVE GROWTH RESULTS OF A VITAMIN B AND G ASSAY OF DRY WHEY, SAMPLE K.

the rice polish was furnished, must be attributed to their relatively low vitamin "B" content. A review of the results obtained from the supplemented and unsupplemented rations with which graduated levels of the test substance were fed, permits an estimation of the relative amount of vitamin "B" and "G" in any single sample, or a comparative evaluation of these factors in the different samples.

An analysis of the data indicate that there is no substantial difference in the amount of vitamin "G" in the dry skim milk and in the dry whey sample "K"; dry whey sample "P" possibly contains slightly less of this factor. The results show, however, that there was a marked variation in the amount of vitamin "B" contained in these products. There was substantially a complete absence of this factor in dry whey sample "P" as shown by the prevalence of paralysis, comparative lack of growth, and early death of all animals receiving this product unsupplemented with rice polish. The dry skimmed milk and the dry whey sample "K" are indicated as having a slightly greater amount of vitamin "B" than dry whey sample "Ko."

Chart V shows the results obtained by superimposing the test products on the basal ration supplemented with rice polish after a period of suppressed growth caused by the elimination of all vitamin "G" with the exception of the small amount required for maintenance and as carried by the 2 per cent rice polish. The growth response resulting from the test products introduced after the period of suppressed growth corroborates the data indicating the amount of vitamin "G" in these products as determined by feeding without previous suppression of growth. (Charts 1, 2, 3 and 4.)

From the various data it is possible to estimate within limits the amount of certain of these products necessary to supply the vitamin "G" require-

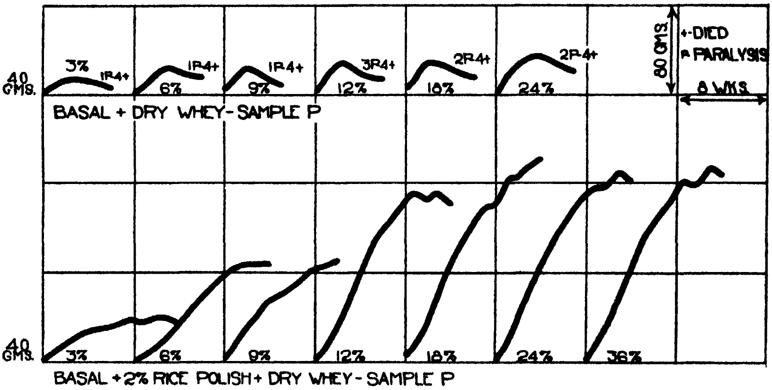

CHART 3. COMPARATIVE GROWTH RESULTS OF A VITAMIN B AND G ASSAY OF DRY WHEY, SAMPLE P.

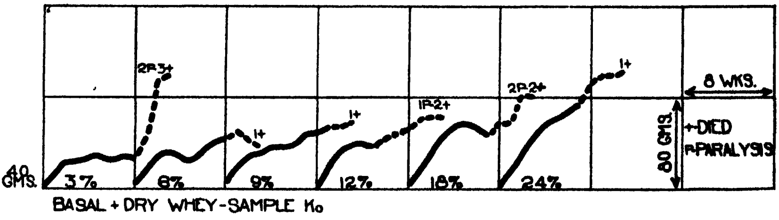

CHART 4. COMPARATIVE GROWTH RESULTS OF A VITAMIN B AND G ASSAY OF DRY WHEY, SAMPLE KO.

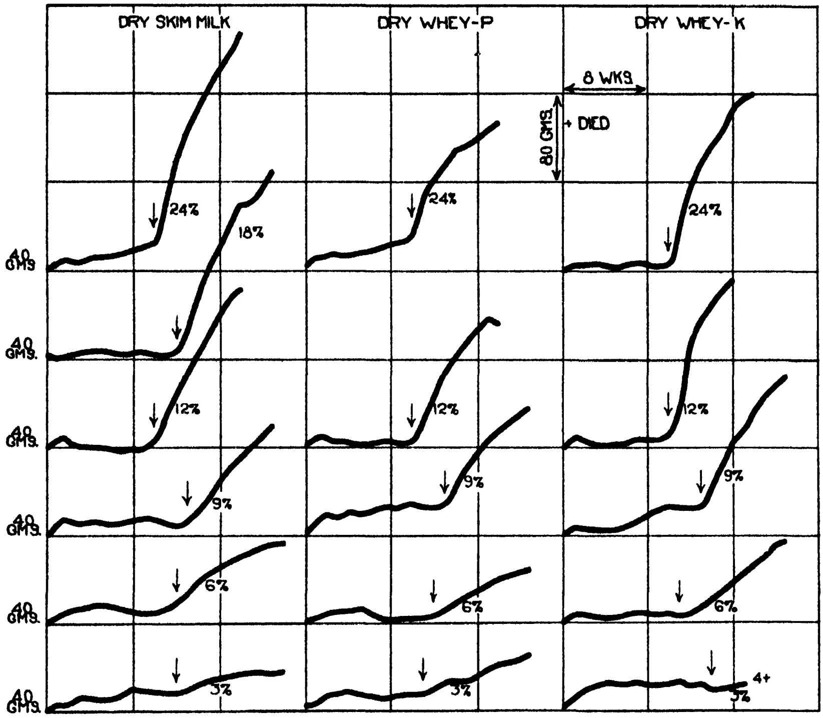

Chart 5. Comparative Growth Results of a Vitamin G Assay of Dry Skim Milk and Dry Wheys, After a Preliminary Period of Suppressed Growth. The Basal Ration Contained 2% Rice Polishings.

ment for normal or optimum growth. It appears that approximately 12 per cent dry skimmed milk or dry whey when supplemented with vitamin "B" supplies a sufficient amount of vitamin "G" for normal growth. On the basis of calculations made from the food consumption records, this is equivalent to approximately 12 to 15 cc. of fluid skimmed milk or whey per day. The data indicate, however, that none of the products, even when fed at the highest levels, furnish a sufficient amount of vitamin "B" for optimum growth. It has been noted by Supplee *et al.* (6) that either a greater amount of vitamin "B" is required at and beyond the period of adolescence than for the earlier periods of structural growth, or that some factor other than vitamin "B" and "G" as now defined is required at the later stages of the life cycle. The data recorded in this report would seem to again call attention to this point. Outhouse and coworkers (1) have indicated, however, that the flattening of the growth curve at this period may be due to the increased vitamin "B" requirement determined primarily by the increasing size of the animal.

SUMMARY

1. The growth promoting properties of the vitamin "G" content of Just roller process dry skimmed milk and spray dried whey are not substantially different.

2. There was a marked variation in the vitamin "B" content of the dry skimmed milk and dry wheys used in these experiments. It is probable that the differences in the treatment during commercial production of these various products accounts for the variations observed.

3. The vitamin "B" content of all of the desiccated products used in this study was relatively lower than the vitamin "G" content. The results indicate that the nutritive value of each of these products is greatly enhanced when supplemented with the vitamin "B" of rice polish, and unquestionably by other sources of the antineuritic factor.

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ZINC CONTENT OF MILK

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INTRODUCTION

Others have shown that the principal foods from both vegetable and animal sources usually contain small amounts of zinc. Giaya (1), Nakaseko (2), and Lehman (3) reported the constant presence of zinc in the liver of both humans and animals. Birckner (4), Hiltner and Wichman (5), and Weitzel (6) found small amounts of zinc in foods.

In so far as the authors could learn, very little information is available as to the zinc content of milk. Birckner (4) found the average amount in cows' milk to be 2.0–5.4 mgms. per kilo. He observed that the maximum amount was present in the milk immediately after parturition. His conclusions are based on 15 determinations made on 14 cows, 7 of which were 70 to 296 days after parturition, 6 the time after parturition was not known and 1 was at the end of lactation. The authors (7) determined the zinc content of cows' milk during the colostrum period.

Only a few studies have been carried out with human milk. Birckner reports 3 determinations on milk from 4 to 15 days after parturition in which a zinc content of 5.66 to 13.78 mgms. per kilo was found. There was a conspicuous increase with time, a result which was quite contrary to that obtained with cows' milk. Nakaseko (2) found 1.2 mgms. per kilo, but the time after parturition was not given.

No record was found of the zinc content of the milk of sheep.

The lack of information as to the zinc content of milk, as shown above, was supplemented by the study reported in this paper in which a slight modification of Birckner's (4) method for the estimation of small amounts of zinc in biological material was used.

MILKS ANALYZED

The human milk was obtained chiefly from 9 women from the gynecology department of the Hokkaida Imperial University. Twenty-five samples were taken 3 to 31 days after parturition, and 3 one year after parturition.

The cows' milk was obtained from 11 head (2 Ayrshire, 6 Holstein, 3 Guernsey) bred at the first and second farm of the Agricultural Department of the University. The samples were taken immediately after parturition and after 3, 4, 24, 48, 72, 96, 120, and 144 hours and from then on at convenient intervals during the lactation period.

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Sheep's milk was tested from 8 head of Ostfiethland sheep of the Tsukissao sheep farm of the Department of Agriculture and Forestry. The samples were collected daily during the early stages of lactation and from then on at monthly intervals. The pH value, specific gravity, and general composition of the milk was also determined.

METHOD OF ESTIMATING ZINC

Previously Birckner's (4) method was chiefly used in estimating the small amounts of zinc which occur in biological materials. The method was improved by using the nephelometric procedure of Breyer (8). The procedure is essentially as follows: the material is ashed, the ash dissolved in HCl solution; the zinc is precipitated as the sulphide by H_2S gas, and the zinc sulphide is collected on a filter. After washing, the zinc sulphide is dissolved in HCl solution and potassium ferrocyanide is added. The turbidity, due to the precipitated zinc ferrocyanide, is compared with standards prepared by using known amounts of zinc.

Unfortunately under the above condition some iron and manganese are precipitated as sulphides with the zinc. Consequently for an accurate determination of zinc in milk the removal of iron and manganese is necessary.

In order to overcome these errors and still use the nephelometric procedure of estimation with Birckner's method, the authors removed the iron, copper, and manganese by precipitation.

METHOD

The material is thoroughly ashed and 0.5 to 1.0 gram of the ash is dissolved in 2 N. HCl. The solution is heated to boiling and ammonium hydroxide is added to precipitate the iron, and ammonium sulphide is added to precipitate the manganese. Under these conditions (9) it is possible to precipitate manganese sulphide without precipitating zinc sulphide. After cooling, the precipitate is removed by filtration and the filtrate is made slightly acid to methyl orange with HCl. Powdered sodium carbonate is added until the solution presents a yellow color and the red color is restored by adding formic acid, drop by drop. The solution is heated to boiling, cooled and treated with H_2S gas for 24 hours.

The precipitate of zinc sulphide is collected on a small filter paper and after washing the precipitate with hydrogen sulphide saturated water, the zinc sulphide is dissolved with 6 cc. of hot 15 per cent HCl solution. After washing with boiling water, the solution is transferred to the measuring tube, and the nephelometric estimation is carried out by a comparison with standard tubes containing known amounts of zinc. The precipitation is produced by adding 5 cc. of potassium ferrocyanide and shaking.

TABLE 1

Cow's milk

Zinc content and general composition

BREED	DAYS AFTER PARTURITION	pH	SPECIFIC GRAVITY	TOTAL SOLIDS	TOTAL PROTEIN	FAT	ASH	ZINC IN 1000 CC. MILK
				%	%	%	%	*mgms.*
Ayrshire 7th parturition	0	6.31	1.0680	30.00	17.29	9.05	1.21	8.27
	2	6.42	1.0370	16.02	4.49	6.97	0.86	3.82
	3	6.37	1.0375	13.94	4.67	6.46	0.78	3.09
	4	6.40	1.0370	14.15	3.54	5.17	0.78	4.33
	5	6.23	1.0380	14.84	4.33	5.76	0.76	3.22
Holstein 197 5th parturition	0	6.27	1.0375	17.36		5.57	1.00	13.03
	2	6.52	1.0330	14.74	5.99	5.13	0.86	6.85
	3	6.38	1.0340	15.30	4.75	5.37	0.84	
	4	6.33	1.0330	16.60	3.50		0.80	5.04
	5	6.46	1.0320	15.30	3.95		0.68	5.04
	6	6.15	1.0320	14.56	3.30		0.72	
	7	6.49	1.0320	14.34	3.38		0.78	4.61
Holstein 239 1st parturition	0	6.24	1.0590	19.86	11.87	3.70	0.98	4.78
	2	6.44	1.0485	16.94	6.84	5.38	0.86	5.23
	3	6.33	1.0380	14.70	5.14	4.67	0.88	3.89
	4	6.34	1.0340	13.90	4.99		0.80	3.22
	5	6.32	1.0305	14.06	5.64	5.36	0.80	3.45
	6	6.33	1.0340	13.64	3.50	6.12	0.78	4.04
	7	6.45	1.0325	13.81	3.36	5.28	0.70	
Holstein 243 1st parturition	0	6.36	1.0567	12.28	14.18	3.50	1.03	12.31
	2	6.41	1.0360	16.48	8.54	4.40	0.90	6.47
	3	6.33	1.0320	13.82	4.83		0.81	4.37
	4	6.47	1.0350	13.08	4.69	4.81	0.86	5.51
	5	6.47	1.0340	13.50	4.32	5.84	0.74	4.41
	6	6.72	1.0350	13.62	4.90	4.59	0.76	4.60
	7	6.48	1.0325	12.80	3.88	4.62	0.70	3.33
Guernsey 168 6th parturition	0		1.0710	30.81	16.81	6.21	0.80	18.66
	2		1.0390	14.54	4.63	5.56	0.84	6.13
	3		1.0350	13.47	3.43	6.06	0.81	6.11
	4		1.0340	14.59	4.03	4.46	0.87	5.14
	5		1.0345				0.86	5.00
Guernsey 189 1st parturition	0		1.0560	27.40	11.11	8.88	0.97	10.95
	2		1.0360	16.14	4.87	5.88	0.72	4.32
	3		1.0280	16.06	3.75	5.63	0.76	4.30
	4		1.0340	14.63	3.13	5.51	0.87	4.73
	5		1.0340	15.31	4.11	5.63	0.81	3.89
	6		1.0340	15.09	4.67	5.19	0.70	3.77
	7		1.0350	15.33	4.41	4.71	0.73	
Guernsey 171 1st parturition	0	6.32	1.0440	19.58	10.91	5.63	1.01	10.41
	4 hrs.	6.44	1.0430	18.73	9.73	4.05	0.86	6.12
	2 days	6.43	1.0305	15.17	4.40	5.51	0.85	4.91
	45	6.52	1.0320	13.35	4.46	4.14	0.73	2.35
	65	6.31	1.0310	13.46	3.31	4.46	0.75	2.19
	90	6.70	1.0310	13.36	3.65	4.80	0.74	2.16
	150	6.41	1.0290	13.12	3.51	4.51	0.64	3.30
	180		1.0287	14.24	3.81	5.62	0.66	3.18
	210	6.39	1.0280	14.92	3.44	6.43	0.73	2.41
	240	6.26	1.0285	16.30	3.48	7.76	0.66	2.32
	300	6.43	1.0315	15.81	4.18	6.55	0.75	3.33
	330	6.36	1.0337	16.14	4.30	7.11	0.77	4.45

TABLE 1 (*Continued*)

BREED	DAYS AFTER PARTURITION	pH	SPECIFIC GRAVITY	TOTAL SOLIDS	TOTAL PROTEIN	FAT	ASH	ZINC IN 1000 CC. MILK
				%	%	%	%	*mgms.*
Holstein 237 3rd parturition	0		1.0440	21.61	8.54	6.92	0.93	14.19
	3 hrs.		1.0430	16.67	6.85	5.05	0.93	6.61
	30 days		1.0324	13.17	3.42	4.68	0.61	2.26
	60		1.0310	12.48	3.51	4.15	0.63	2.46
	90		1.0300	12.74	3.77	4.17	0.61	1.72
	120		1.0280	12.04	3.22	4.36	0.66	2.26
	150	6.23	1.0290	13.13	4.14	4.50	0.70	2.51
	180	6.28	1.0320	13.74	3.82	4.26	0.75	2.45
	210	6.41	1.0295	13.20	3.27	4.88	0.74	2.86
	240	6.35	1.0290	12.99	3.55	4.71	0.72	3.46
	300	6.41	1.0341	12.83	3.79	3.36	0.78	3.54
	330	6.43	1.0310	13.30	3.88	4.54	0.72	3.08
	360	6.21	1.0330	13.20	3.79	4.13	0.59	3.21
	420		1.0297	14.13	3.85	5.03	0.59	2.99
	480		1.0323	12.09	3.74	3.04	0.54	3.69
	510		1.0320	13.14	3.76	4.19	0.66	3.33
Holstein 195 3rd parturition	0		1.0694	23.12	14.46	3.52	0.86	16.72
	30		1.0330	13.10	4.15	5.03	0.81	3.80
Ayrshire 71 3rd parturition	Last milk of lactation	6.61	1.0331	14.77	4.25	5.56	0.78	4.65
Holstein 1st parturition	Last milk of lactation	6.68	1.0305				0.82	5.41

STANDARD AND STOCK SOLUTION

Weigh accurately 1.2447 grams of pure zinc oxide, dissolve in HCl and add 100 cc. of distilled water; neutralize the solution with dilute ammonium hydroxide until the solution becomes transparent. Make up to a volume of one liter in a volumetric flask, using distilled water. This is the stock solution. Dilute 10 cc. of the stock solution to 100 cc. to use as a standard solution, which contains 0.1 mgm. of zinc per cc.

RESULTS

Table 1 gives the amount of zinc found in cows' milk, table 2 sheep's milk, table 3 human milk, and table 4 the average amount of zinc in human, cow and sheep milk as influenced by the period of lactation.

In addition to the data given in these tables, the following amounts of zinc were found in the milk from normal individual cows: 3.65, 3.29, 2.89,

TABLE 2

Sheep milk

Zinc content and general composition

AGE & PARTURITION	DAYS AFTER PARTURITION	PH	SPECIFIC GRAVITY	TOTAL SOLIDS	TOTAL PROTEIN	FAT	ASH	ZINC IN 1000 CC. MILK
				%	%	%	%	*mgms.*
4 years old 1st parturition	0	6.63	1.0550		15.36	18.80	1.14	11.85
	2	6.56	1.0420	20.11	8.29	7.68	0.99	4.72
	3	6.65	1.0400	17.73	6.23	7.48	0.89	4.47
	4	6.59	1.0380	19.69	6.50	8.20	0.82	3.94
	7	6.66	1.0380	18.21	6.83	7.28	0.71	3.39
	1 mo.	6.59	1.0380	16.98	4.91	6.82	0.69	2.64
	2 "	6.48	1.0350	15.38	4.48	6.28	0.70	2.51
	3 "	6.50	1.0400	17.40	5.34	6.41	0.82	2.27
	4 "	6.38	1.0370	18.04	5.59	6.35	0.78	2.31
	5 "	6.43	1.0370				0.71	2.13
	6 "		1.0380	19.75	5.99	9.75	0.48	2.48
	7 "	6.54	1.0395	21.48	5.90	9.16	0.93	2.51
	8 "	6.65	1.0440	21.02	7.66	8.32	0.95	3.17
	Last milk of lactation	6.65	1.0415	22.88	6.04	9.53	0.83	2.95
4 years old 1st parturition	0	6.36	1.0620	26.34	13.12	9.88	0.92	14.72
	3	6.62	1.0380	19.42	7.64	7.85	0.92	3.43
	4	6.63	1.0310	18.87	5.68	7.83	0.90	3.38
	8	6.49	1.0380	19.95	5.32	6.72	0.89	3.06
	9	6.52	1.0360	16.28	4.68	5.87	0.82	2.52
	10	6.75	1.0350	16.78	4.80	6.44	0.82	3.11
	1 mo.	6.53	1.0350	16.15	4.52	6.29	0.76	3.16
	2 "	6.32	1.0355	16.38	4.54	7.24	0.72	2.96
	3 "	6.62	1.0370	17.72	5.29	6.33	0.75	2.81
	4 "	6.48	1.0366	17.25	5.18	6.17	0.72	2.86
	5	6.11	1.0376				0.78	4.48
	6		1.0366	16.16	7.56	7.19	0.96	2.82
	7	6.44	1.0350	22.72	6.71	10.33	0.81	3.00
	8		1.0390	18.78	7.28	7.04	0.84	3.39
	Last milk of lactation	6.37	1.0408	28.86	28.86	14.21	1.08	3.36
4 years old 2nd parturition	1 mo.	6.53	1.0376	17.32	4.19	7.27	0.80	2.77
	2 "	6.39	1.0360	16.04	4.85	5.87	0.81	2.37
	3 "	6.55	1.0370	17.73	5.81	6.98	0.78	2.50
	4 "	6.33	1.0385	17.26	5.49	5.47	0.88	2.21
	5 "		1.0370	17.89			0.84	2.67
	6 "	6.43	1.0440	17.11	6.03	9.03	0.80	2.68
	7 "	6.49	1.0422	19.32	7.08	8.24	1.06	2.91
	8 "	6.78	1.0465	25.60	9.42	10.33	1.04	3.05
5 years old 2nd parturition	1 mo.	6.55	1.0350	15.63	3.63	8.14	0.70	2.93
	2 "	6.62	1.0360	16.60	5.78	6.08	0.72	2.92
	3 "		1.0370	15.92	5.26	5.27	0.77	3.72
	4 "	6.52	1.0370	14.90	4.83	4.94	0.72	1.98
	5 "		1.0370	16.69	5.44	9.71	0.77	4.18
	6 "	6.57	1.0379	18.61	5.73	7.22	0.84	3.02
	7 "	6.40	1.0443	25.59	8.08	13.71	1.09	2.65
	8 "	6.80	1.0437	20.92	7.94	9.64	0.97	3.05
	Last milk of lactation	6.25	1.0300	17.24	6.63	7.68	0.85	3.39

TABLE 2 (*Continued*)

AGE & PARTURITION	DAYS AFTER PARTURITION	pH	SPECIFIC GRAVITY	TOTAL SOLIDS	TOTAL PROTEIN	FAT	ASH	ZINC IN 1000 CC. MILK
				%	%	%	%	*mgms.*
5 years old 2nd parturition	1 mo.	6.59	1.0340	13.92	3.58	6.53	0.71	3.12
	2 "	6.48	1.0350	13.35	3.70	6.70	0.74	2.79
	3 "	6.66	1.0370	15.48	5.48	5.77	0.87	3.71
	4 "	6.29	1.0350	15.94	4.77	6.01	0.68	2.34
	5 "		1.0390	16.68	4.07	8.21	0.82	2.64
	6 "	6.61	1.0411	18.03	6.10	8.31	0.83	3.19
	7 "		1.0434	19.63	7.26	8.28	0.81	2.67
	Last milk of lactation		1.0420	20.11	7.42	7.78	0.95	3.18
4 years old 1st parturition	1 mo.	6.30	1.0360	17.77	5.63	5.58	0.76	3.48
	2 "	6.05	1.0365	16.96	4.91	5.88	0.73	3.22
	3 "	6.38	1.0360	17.68	5.31	6.40	0.75	3.26
	4 "	6.43	1.0360	17.32	5.22	6.38	0.76	2.90
	5 "		1.0370	17.89			0.84	3.58
	6 "	6.42	1.0380	17.32	5.49		0.81	3.40
	7 "	6.46	1.0376	22.50	6.74	10.41	1.03	4.08
	Last milk of lactation	6.65	1.0415	22.80	8.68	9.58	0.83	3.64
4 years old 1st parturition	1 mo.	6.48	1.0370	16.65	5.15	5.70	0.82	2.84
	2 "	6.31	1.0370	15.90	4.66	5.67	0.72	1.92
	3 "	6.38	1.0360	17.39	4.95	7.16	0.86	2.91
	4 "	6.38	1.0360	17.96	5.23	6.35	0.68	2.60
	5 "	6.61	1.0370	18.04	5.59	6.35	0.78	2.62
	6 "	6.40	1.0402	15.82	5.49	5.57	0.85	2.27
	7 "	6.40	1.0401	19.64	6.46	7.90	0.87	3.06
	8 "	6.66	1.0699	20.04	6.64	7.40	0.86	2.37
	Last milk of lactation	6.10	1.0355	18.91	9.96	7.85	0.98	3.68
4 years old 1st parturition	0	6.29	1.0609	30.22	16.94	10.16	0.92	14.42
	1 mo.	6.47	1.0360	16.33	5.02	5.98	0.65	2.41
	2 "	6.36	1.0355	15.76	4.34	5.91	0.75	3.10
	3 "	6.66	1.0375	17.26	4.81	6.74	0.82	2.81
	4 "		1.0360	17.28	5.51	6.54	0.72	1.94
	5 "	6.40	1.0491	19.84	6.46	7.90	0.87	2.19
	6 "		1.0390	19.81	6.68	10.78	0.99	2.46
	7 "	6.51	1.0340	19.86	6.42	9.40	0.87	2.37
	8 "	6.62	1.0344	19.13	6.75	8.09	0.96	2.84

2.00, and 3.33 mgms. per 1,000 cc. Samples of normal mixed herd milk gave 3.46, 2.94, 3.27, 4.12, 3.12, 3.15, and 3.33 mgms. per 1,000 cc. of milk.

Contrary to Birckner's three determinations of 5.66 mgms. at 4 days, 11.5 mgms. at 9 days and 13.78 mgms. at 15 days, the results presented here show that the zinc content of human milk decreases fairly definitely during the first few days after parturition. The greatest decrease in zinc takes place during the first few days of lactation; after about 10 to 15 days the zinc content becomes nearly constant and continues at about the same

TABLE 3

Zinc content of human milk

EXPERIMENT NO.	TIME AFTER PARTURITION	ZINC IN 1000 CC. OF MILK
	days	*mgms.*
I	3	7.35
	5	7.40
	7	6.46
II	4	4.43
	11	5.33
III	4	5.80
	6	4.54
	8	6.16
	10	3.70
	15	3.09
	16	2.86
	24	2.78
	25	1.23
	30	1.76
	32	1.60
IV	9	5.17
	10	3.72
V	6	6.35
	7	6.20
VI	10	4.89
	14	4.44
	15	4.03
VII	28	3.03
	30	3.63
VIII	360	3.07
	381	2.89
IX	360	3.84

level throughout the remainder of the lactation period, with perhaps a possible increase at the end of lactation.

The zinc content of the milk of cows and sheep is about the same as that of human milk. Also the zinc content was quite high immediately after parturition, falling to a relatively constant value in about 10 days. An increase in the zinc content at the end of lactation was noted, particularly in the case of cows' milk, as shown particularly by the last two values in table 1.

TABLE 4

Influence of stage of lactation upon zinc content of milk

HUMAN		COW		SHEEP	
TIME AFTER PARTURITION	ZINC IN 1000 CC. OF MILK	TIME AFTER PARTURITION	ZINC IN 1000 CC. OF MILK	TIME AFTER PARTURITION	ZINC IN 1000 CC. OF MILK
days	*mgms.*	*hours*	*mgms.*	*days*	*mgms.*
3	7.35	0	13.57	0	13.78
4	5.12	3	6.61	2	4.71
5	6.34	4	6.12	3	3.90
6	5.03	days		4	3.36
7	6.18	2	5.31	7	3.18
8	6.71	3	4.35	8	3.06
9	5.19	4	4.68	9	3.04
10	4.42	5	4.16	10	3.11
11	5.33	6	4.18	months	
14	4.44	7	4.19	1	2.89
15	3.56	months		2	2.81
16	2.86	1	2.29	3	2.83
24	2.78	2	2.35	4	2.45
25	1.23	3	2.12	5	2.69
28	3.03	4	2.28	6	2.74
30	2.67	5	3.07	7	2.91
32	1.60	6	3.00	8	2.97
1 year	3.25	7	2.63	last	3.21
		8	2.89		
		10	3.44		
		11	3.76		
		12	3.21		
		14	2.99		
		16	3.69		
		17	3.33		

SUMMARY

1. A modification of Birckner's method for the determination of zinc was developed whereby the interfering iron and manganese were removed.

2. The zinc content of human, cow, and sheep's milk was determined at various intervals throughout the lactation period.

3. Little difference was noted in the zinc content of human, cow, and sheep's milk.

4. A marked decrease in the zinc content of milk was noted during the first few days of the lactation period. During the middle normal portion of the lactation period the zinc content of the milk was about constant, but increased in amount at the end.

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MANGANESE CONTENT OF MILK

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INTRODUCTION

The existence of manganese in blood and animal tissue has been known for a long time. Warzer (1) in 1830 reported its existence in blood. Bertrand (2) in 1905 found that minute amounts of manganese affected the growth of bacteria exerting a catalytic function. Bradley (3) in 1907 found manganese in the tissue and eggs of shell fish in fresh water. Dumetrescou and Nicolau (4) in 1910 determined the manganese content of fishes and hen's egg. Bertrand and Medigreceanu (5) in 1912 observed the existence of manganese in normal blood. They (6) also investigated the manganese content of the tissues of invertebrates, fishes, and lower animals, as a result of which they concluded that manganese played a very important rôle in growth and functions of animals. Reiman and Minot (7) in 1920 who made quite a study of the manganese content of human blood and tissues, pointed out that with but few exceptions, there was a constant level of manganese in the blood. Its presence in tissues is confined largely to the liver as in other animals.

Manganese in milk has received but little attention. Dumetrescou and Nicolau (4) determined manganese in milk and Bertrand and Medigreecianu (6) showed that milk contained a small amount of manganese. Nakaye (8) after studying the manganese content of plants and animals, turned his attention toward milk. He determined the manganese content of normal and colostrum milk, being interested in whether the manganese content of the milk was affected by the growth of the calf. Unfortunately his method was not adequate for milk.

The present paper deals with the authors' study of manganese in milk. It was found that the previously used analytical methods for the determination of manganese in biological material were unsuited to milk. Not only because of an error in the methods but because too much material was required. (Nayake found that 1000 cc. was not sufficient.) The first step therefore was the development of a method which was suitable for milk.

METHOD FOR MANGANESE

The methods (4), (5), (7), (9), (10), (11) hitherto developed for the determination of manganese in biological material are quite varied. The ordinary colorimetric method is most often used.

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In applying this method to milk the color is interfered with by the formation of a precipitate with the chloride and phosphate radical contained in the milk. This interference was avoided by heating with concentrated nitric acid. By a quantitative relation between the amount of potassium persulphate and silver nitrate added an accurate determination of manganese could be made using small amounts of materials to be tested.

Method. The sample was ashed at dull redness until the carbon was completely removed. The ash (1.0–2.0 grams) was then treated with 15 cc. of a 50 per cent solution of nitric acid and left for 24 hours. It was then heated gently on a sand bath until it was thoroughly dry. After cooling the residue was well moistened by adding 5 cc. of 2 N. nitric acid and 5 cc. of distilled water. The solution was warmed on a sand bath for half an hour and when digestion was complete it was filtered with thorough washing into a volumetric flask. To the filtrate 0.2 cc. of a 2 per cent solution of silver nitrate and 0.2 grams of potassium persulphate were added. The solution was then heated gently on a sand bath until the maximum color had developed. The depth of color was then compared with that of a standard solution of potassium permanganate, using a Leitz colorimeter.

Standard stock solution. 2.877 grams of pure potassium permanganate were dissolved in water and a little sulphurous acid was added to discharge the color of the potassium permanganate. The solution was made up to a volume of 1000 cc. Each cc. of this solution contains 1.0 mgm. of manganese.

TABLE 1

Recovery of manganese added to milk

0.01 or 0.02 mgm. of manganese used for color standard

	VOLUME OF MILK SAMPLE TAKEN FOR ASHING	WEIGHT OF ASH TAKEN FOR ANALYSIS	AMOUNT OF MANGANESE ADDED TO MILK	TOTAL MANGANESE FOUND	MANGANESE RECOVERED
	cc.	*grams*	*mgms.*	*mgms.*	*mgms.*
Mixed milk		1.0	0	0.0066	
(colostrum)		1.0	0.01	0.0167	0.010
“	185	1.5	0	0.0115	
	185	1.5	0.01	0.0215	0.0100
“	162	1.2	0	0.0088	
	162	1.2	0.01	0.0181	0.0093
Mixed milk	194	1.5	0	0.0056	
(normal)	194	1.5	0.01	0.0151	0.095
“	194	1.5	0	0.0056	
	194	1.5	0.02	0.0266	0.0210
Individual	200		0	0.0060	
(normal)	200		0.01	0.0147	0.087

TABLE 2

Cows' milk (Colostrum)

Manganese content and general composition

BREED	TIME AFTER PARTURITION	pH	SPECIFIC GRAVITY	COLOR			TOTAL SOLIDS	TOTAL PROTEIN	ASH	FAT	MANGANESE IN 1000 CC.
				Red	Yellow	Blue					
	hours						%	%	%	%	*mgms.*
Ayrshire 86 5th parturition	0	6.38	1.0510	0.4	0.4		22.48	10.14	8.70	1.06	0.161
	24	6.28	1.0365	0.3	0.4	0.4	18.02	5.65	5.33	0.90	0.069
	48	6.41	1.0320	0.4	0.2	0.6	15.88	4.98	4.37	0.86	0.060
	72	6.44	1.0320	0.2	0.2	0.3	14.58	3.94	4.77	0.74	0.056
	96	6.45	1.0345	0.3	0.3	0.3	13.52	3.71	4.44	0.86	0.065
Ayrshire 115 1st parturition	0	6.45	1.0460	0.4	0.4	0.1	22.78	9.96	8.56	1.04	0.210
	24	6.52	1.0395	0.4	0.2	0.4	16.04	5.19	5.19	0.90	0.057
	72	6.51	1.0360	0.2	0.2	0.3	11.70	3.83	4.30	0.76	0.045
	96	6.57	1.0330	0.3	0.4	0.7	13.73	3.92	4.26	0.88	0.060
Holstein 173 8th parturition	0	6.27	1.0567	0.5	0.6	0.1	22.20	10.65	7.81	0.91	0.154
	24	6.39	1.0320	0.5	0.4	0.3	16.70	4.77	6.55	0.74	0.083
	48	6.44	1.0340	0.2	0.4	0.2	13.42	4.16	4.75	0.70	0.046
	72	6.22	1.0320	0.6	0.4	0.4	12.82	4.25	4.00	0.72	0.056
	96		1.0330	0.3	0.4	0.3	13.36	3.51	4.90	0.78	0.053
Holstein 249 1st parturition	0	6.31	1.0650	0.8	0.6	0.2	26.38	16.24	6.69	1.02	0.205
	24	6.48	1.0345	0.8	0.4	0.6	14.46	5.01	4.30	0.82	0.060
	48	6.44	1.0345	0.7	0.3	0.6	13.76	4.61	3.91	0.78	0.062
	72	6.31	1.0320	0.3	0.3	0.2	13.36	3.96	4.99	0.62	0.047
Guernsey 171 1st parturition	0	6.41	1.0650	0.8	0.5	0.3	20.46	13.73	5.92	0.98	0.104
	24	6.38	1.0400	0.6	0.4	0.3	16.02	5.89	5.72	0.97	0.057
	48	6.62	1.0355	0.6	0.3	0.2	14.30	4.13	5.37	0.81	0.068
	96	6.40	1.0330	0.3	0.4	0.4	13.55	4.52	5.11	0.76	0.076

Standard working solution. 5 cc. of the above stock solution were diluted to 500 cc. with distilled water. Each cc. of this solution contains 0.01 mgms. of manganese.

Results obtained when this method was applied to milk to which known amounts of manganese were added are given in table 1. This table shows that the method is quite reliable when it comes to determining the amount of added manganese.

SOURCES OF MILK SAMPLES

Cows' Milk. The milk from 2 Holstein, 2 Ayrshire and 1 Guernsey cows which were bred at the first and second farms of the Hokkaido Imperial University was tested for manganese. The milk was examined immediately after parturition and on the 2nd, 3rd, 4th, and 5th days after parturition. The manganese content of normal milk was determined by examining mixed milk and milk from individual cows.

Sheep milk. Samples were obtained from 4 sheep which were bred at the Tsukisappu sheep farm of the Department of Agriculture and Forestry. The samples were obtained at 30 day intervals.

Horse milk. Samples of normal horse milk were obtained from the veterinary hospital of the Hokkaido Imperial University.

RESULTS

Table 2 shows the manganese content of cows' milk during the first few days after parturition, and table 3 of normal milk. Tables 4 and 5 show the manganese content of sheep and horse milk, respectively. Table 6 gives the maximum, minimum and average manganese content of the milk from the different animals under the various conditions.

TABLE 3

Cows' milk (Normal)

Manganese content and general composition

NO. OF EXPERIMENT	BREED	SPECIFIC GRAVITY	TOTAL SOLIDS	TOTAL PROTEIN	FAT	ASH	MANGANESE IN 1000 CC.
I	Holstein Individual	1.0297	13.30	3.88	4.54	0.72	0.036
	"	1.0340	12.83	3.79	3.76	0.78	0.039
I	Mixed	1.0280	13.00	3.85	3.65	0.71	0.031
II	"	1.0310	12.41	3.41	3.50	0.73	0.026
III	"	1.0300	12.69	4.02	3.79	0.66	0.048
IV	"	1.0285	13.11			0.71	0.019
V	"	1.0290	13.07	4.05	3.66	0.70	0.031
VI	"	1.0287	12.60	4.00	3.77	0.66	0.033

TABLE 4

Sheep milk

Manganese content and general composition

AGE AND PARTURITION	TIME AFTER PARTURITION	pH	SPECIFIC GRAVITY	COLOR			TOTAL SOLID	TOTAL PROTEIN	FAT	ASH	MANGANESE IN 1000 CC.
				Red	Yellow	Blue					
	days						%	%	%	%	*mgms.*
5 yrs. old 2nd parturition	60	6.60	1.0360	0.2	0.1	1.1	16.60	5.78	6.08	0.72	0.050
	90		1.0370	0.7		2.1	15.92	5.26	5.27	0.77	0.051
	150	6.62	1.0370	1.1	0.1	0.7	16.69	5.44	9.71	0.77	0.064
	210	6.57	1.0379	1.0	1.2	2.0	18.61	5.73	7.22	0.84	0.092
	240	6.80	1.0437	0.4	0.5	1.3	20.92	7.94	9.64	0.97	0.084
4 yrs. old 1st parturition	60	6.36	1.0355	0.4	0.1	0.9	15.76	4.34	5.91	0.75	0.046
	90	6.66	1.0375	0.5		0.2	17.26	4.81	6.74	0.85	0.041
	150		1.0360	0.7		2.0	17.48	5.51	6.54	0.72	0.045
	210		1.0390	0.5	0.9	1.0	19.81	6.68	10.78	0.91	0.064
	240	6.62	1.0344	0.3	0.2	1.0	19.13	6.75	8.09	0.96	0.067
4 yrs. old 1st parturition	60	6.35	1.0365	0.1	0.1	0.7	16.96	4.91	5.88	0.73	0.052
	90	6.38	1.0360	0.3		1.2	17.68	5.37	4.40	0.75	0.057
	150	6.43	1.0370	0.1		0.7	17.89		6.36	0.76	0.071
	210		1.0380	0.1		0.5	17.32	5.49		0.81	0.115
4 yrs. old 1st parturition	60	6.31	1.0370	0.2	0.1	0.8	15.90	4.66	5.67	0.72	0.054
	90	6.38	1.0360	0.3		1.2	17.34	4.95	7.16	0.86	0.062
	210	6.01	1.0420	0.1	0.1	0.4	15.85	5.49	5.57	0.85	0.060
	240	6.66	1.0399	0.2	0.1	0.3	20.01	6.64	7.40	0.86	0.077

TABLE 5

Horse milk

Manganese content and general composition

NO. OF EXPERIMENTS	TIME AFTER PARTURITION	SPECIFIC GRAVITY	TOTAL SOLIDS	TOTAL PROTEIN	FAT	ASH	MANGANESE IN 1000 CC.
	days		%	%	%	%	*mgms.*
I	160	1.0105	8.52	2.12	1.60	0.36	0.041
II	162	1.0210	7.81	2.36	1.82	0.39	0.042
III		1.0270	11.41	2.02	2.79	0.46	0.037
IV		1.0251	9.51	3.41	2.50	0.40	0.039

TABLE 6

Minimum, maximum and average manganese content of milk

MAMMAL	DESCRIPTION	TIME AFTER PARTURITION	MANGANESE IN 1000 CC.		
			Minimum	Maximum	Average
		days	*mgms.*	*mgms.*	*mgms.*
Cows' milk	Colostrum	0	0.10	0.21	0.16
		1	0.05	0.08	0.07
		2	0.05	0.06	0.06
		3	0.04	0.05	0.05
		4	0.05	0.06	0.06
	Normal (Individual)		0.03	0.04	0.03
	(Mixed)		0.02	0.04	0.03
Sheep milk		60	0.04	0.05	0.05
		90	0.04	0.06	0.05
		150	0.04	0.07	0.06
		210	0.06	0.11	0.09
		240	0.06	0.08	0.07
Horse milk	Normal		0.03	0.04	0.04

Tables 2 and 6 show quite clearly that the first colostrum milk after parturition contains proportionally a much larger amount of manganese than does the normal milk. The amount of manganese being much less the second day. During the first 4 days after parturition the milk contains about twice as much manganese as does normal milk.

Sheep milk as shown by table 4 contains considerably more manganese than cows' milk and the manganese shows a tendency to increase toward the end of the lactation period.

Tables 5 and 6 indicate that normal horse milk may be slightly higher in manganese than normal cows' milk.

SUMMARY

1. A modification of the colorimetric method for the determination of manganese in biological material was developed whereby the interference of the precipitation of chlorides and phosphates were avoided.

2. Colostrum milk (cows') contains much more manganese than does normal milk, the first sample after parturition being especially high in manganese.

3. Sheep milk is higher in manganese than is cows' or horse milk.

4. The manganese content of sheep milk tends to increase toward the end of lactation.

Normal horse milk contains slightly more manganese than does normal cows' milk.

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A BIOCHEMICAL STUDY OF IRRADIATED MILK

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Recently O'Brien, Davis and Armstrong[1] have described a method for irradiating milk with ultraviolet light. Their source of light is an arc from carbon electrodes of special composition and has an energy emission, in the wave length region from 2,800 to 3,100 Ångstrom units, 1,260 times that of bright sunlight. There is very little energy emitted at wave lengths shorter than 2,800 Å. They point out that radiation of wave lengths between 2,800 to 3,100 Å. is very effective in producing anti-rachitic potency, while radiation of wave lengths less than 2,800 Å. is destructive to vitamin A, causes protein coagulation and produces a bad odor and taste in the milk. The apparatus in which the milk is irradiated is essentially a glass cylinder. The carbon arc is in the center of this cylinder and the milk flows down the inside of the cylinder in a thin film in such a way that each particle of milk receives a uniform dosage of light. Feeding experiments which they have conducted show that the vitamin D potency of irratiated milk is increased 16 times, while the vitamin A potency is 86 per cent. of that in the original milk. They also report bacteriological studies showing a decided reduction in the bacterial count after irradiation.

In the present paper a biochemical study of milk and butter from milk irradiated by the above process is reported. Since it was anticipated that effects of irradiation would be slight, the milk used in the following work was given a much longer exposure to ultraviolet light than is the usual practice. This was accomplished by passing it thru the irradiating process eight times. In this way it was hoped that any effects of ultraviolet light would be greatly exaggerated and therefore more easily detected by the methods employed. The samples of certified milk were irradiated at a plant located near Erie, Pennsylvania. Uniform samples of irradiated and non-irradiated milk were cooled, placed in thermos bottles and shipped to our laboratory at State College, arriving the following morning in good condition.

EXPERIMENTAL

Chemical Composition of Milk

The object of this phase of the work was to determine what changes in chemical composition, if any, take place during irradiation. Several sets

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[1] O'Brien, B., Davis, A. G., and Armstrong, E. L. Combined bactericidal and antirachitic effects induced in milk by carbon arc irradiation. Penna. Med. Jour. April, 1931.

of samples were analyzed before and after irradiation. A typical set of analyses is given in table 1. The results are averages of three determinations.

TABLE 1

Showing the Analysis of Milk before and after Irradiation

	BEFORE IRRADIATION	AFTER IRRADIATION
Specific Gravity at 25° C.	1.0271	1.0272
Specific Conductivity	0.0049	0.0046
pH	6.57	6.74
Total Solids	13.28	13.22
Ash	0.69	0.69
Fat	4.40	4.31
Total Protein (N × 6.38)	3.48	3.42
Lactose	4.63	4.62
Sum (Ash, fat, protein, lactose)	13.20	13.04
Casein	2.50	2.47
Albumin	0.73	0.69

It will be seen that there are only slight differences in composition between the two samples. The pH of the irradiated sample is slightly higher than that of the non-irradiated sample. This can be accounted for on the basis of the reduction in bacterial count of the irradiated sample. Since the samples were not analyzed until the day following irradiation, more acidity may have developed in the untreated sample due to its higher bacterial count. Since ultraviolet light is known to cause a coagulation of albumin it was thought that possibly the albumin content of the irradiated sample would be considerably less than that of the untreated sample. The slight difference noted is hardly sufficient to be significant. Some of the difference can be accounted for by the lower total solid and protein content of the irradiated sample. Since the irradiated sample was irradiated eight times, it is certainly safe to say that normal irradiation by the process described does not cause detectable coagulation of the albumin in milk.

Chemical Composition of Butter Fat

In this work two samples of butter were used, one from untreated milk and the other from irradiated milk. The butter samples, of about a pound each, were melted and the butter fat used for the determination of various fat constants. Table 2 gives the results of the analyses. The results are averages of three determinations.

The most significant thing in table 2 is the shorter induction period for oxidation of the butter fat which had been irradiated. Irradiation undoubtedly initiates the oxidation process of the milk fat. It should be pointed out, however, that even after irradiation the sample studied had excellent

TABLE 2

Showing the Analysis of Butter Fat prepared from Milk before and after Irradiation

	BEFORE IRRADIATION	AFTER IRRADIATION
Refractive Index at 40° C.	1.4551	1.4551
Acid Number	0.93	0.96
Saponification Number	224.5	225.4
Acetyl Number	4.81	4.69
Reichert-Meissl Number	28.94	29.64
Polenske Number	1.51	1.89
Iodine Number	37.6	38.3
Kriest Test	Negative	Negative
Induction Period for Oxidation	20 hrs.	17 hrs.
Induction Period after 6 months storage	10 hrs.	8 hrs.

keeping qualities, superior to that of fat obtained from high quality creamery butter which gave an induction period for oxidation of 11 hours. In order to study the effect of irradiation on the keeping quality of butter fat the samples were stored in a refrigerator for six months and the induction period for oxidation determined again. It will be noticed that the induction period in both cases has been reduced about one-half. In other words the rate of deterioration is about the same in both cases.

In considering the fat constants it will be noticed that the changes after irradiation are slight. In every case except the iodine number the results lend themselves to the interpretation that slight oxidation has occurred. The higher acid, saponification, Reichert-Meissl and Polenske numbers of the irradiated sample, indicate a possible cleavage of long chain into short chain fatty acids. The lower acetyl number of the irradiated sample suggests the oxidation of hydroxyl groups. Since it is generally thought that oxidation takes place at the double bonds, one would expect a lower iodine number after irradiation. This is not the case. The higher iodine number after irradiation agrees well with the shorter induction period of this sample since unsaturated fats are known to have a shorter induction period than saturated fats. It is possible that irradiation shortens the induction period due to the introduction of double bonds in the molecule which is possibly a preliminary stage in the oxidation of a fat. Since such slight changes were found in fat exposed to such excessive doses of ultraviolet light, one can conclude that the usual dose would have an insignificant effect on the fat of milk.

Enzyme Studies with Pepsin

The object of this phase of the work was to determine what effect irradiation has on the rate of digestion of the proteins of milk by pepsin. The method used was as follows: 10 cc. of milk were placed in a beaker together with 50 cc. of water and 5 cc. of 1.0 per cent commercial pepsin solution. This was then titrated with standard HCl solution to a pH of 2.0 at 25° C. using the quinhydrone electrode. This titration was made for both the irradiated milk and the control of non-irradiated milk. Next 10 cc. portions of each milk were placed in small Erlenmeyer flasks together with 50 cc. of water. The predetermined amount of standard HCl solution was then added to each flask to bring each solution to a pH of 2.0 after the addition of pepsin. These flasks were then placed in an incubator at 38° C. and allowed to remain there until they had reached a temperature of 38° C. Then 5 cc. of a 1.0 per cent commercial pepsin solution, which had a temperature of 38° C., were added to each flask. The flasks were kept at 38° C. throughout the experiment. At intervals two flasks of irradiated and of non-irradiated milk were removed and treated as follows: the samples were transferred to 200 cc. volumetric flasks and sufficient standard NaOH solution added to neutralize the added HCl solution and sufficient excess to make 14 cc. of N/10 NaOH in each flask. Then 4 cc. of Fehling's $CuSO_4$ solution were added to each flask and the solutions made up to volume. After a thorough mixing the solutions were filtered and 100 cc. portions of each filtrate were analyzed for nitrogen by the Kjeldahl method. The results indicate the amount of non-protein nitrogen present and are a measure of the amount of digestion taking place.

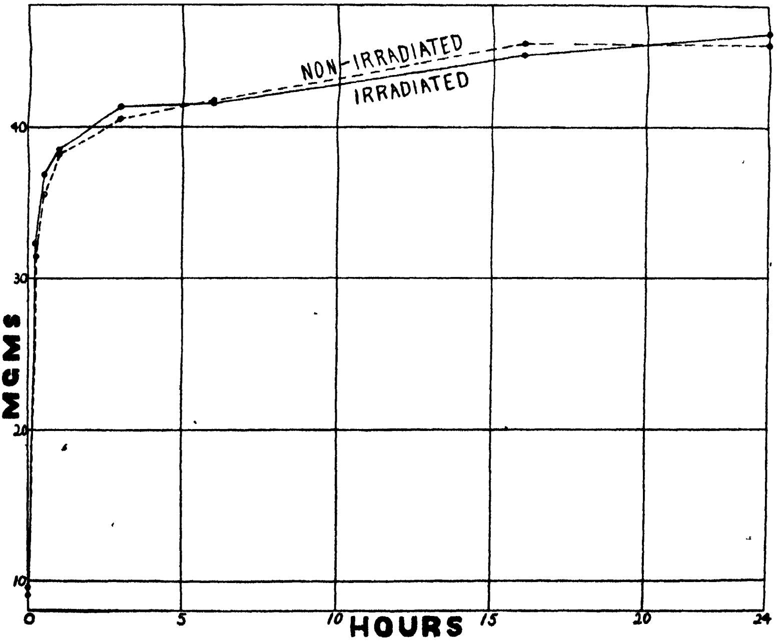

FIG. 1. SHOWING MILLIGRAMS OF NON-PROTEIN NITROGEN IN 10 CC. OF MILK DURING DIGESTION BY PEPSIN AT pH 2.0.

The above experiment was run several times and a typical set of results is given in Fig. 1. Each value is an average of duplicate determinations.

It should be noted that at the beginning of the experiment there was 0.5 mgm. more of non-protein nitrogen in the non-irradiated sample than in the irradiated sample. Hence, one should add 0.5 mgm. to each of the values in the curve for irradiated milk when comparing the two curves. If this were done the irradiated sample would show a greater amount of non-protein nitrogen at each period of analysis except the 16-hour period. Although the differences are not great, it appears that irradiation does not retard the digestion of milk proteins by pepsin but accelerates it slightly, especially during the early stages of digestion. Since the irradiated sample was given a heavy dose of ultraviolet light in this experiment as in the other experiments, it is very likely that normal irradiation by the process described has little effect on the digestibility of milk proteins by pepsin at a pH of 2.0.

Enzyme Studies with Trypsin

This phase of the work was very similar to that of the work with pepsin. The procedure differed in that the samples were brought to a pH of 8.1 with standard NaOH solution and at the end of digestion periods sufficient NaOH solution was added to make the total added equivalent to 14 cc. of N/10 NaOH. The trypsin used was a commercial preparation and 5 cc. of a 0.2 per cent solution were used in each flask.

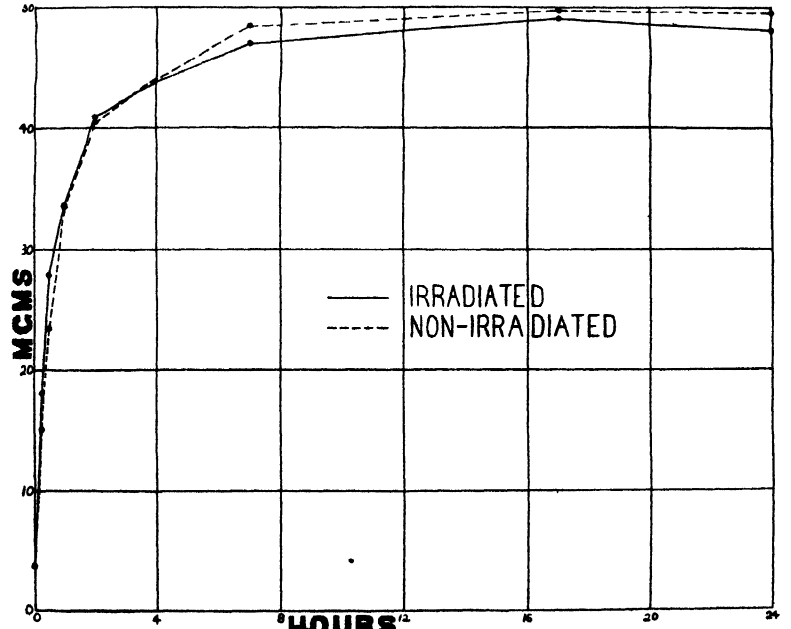

FIG. 2. SHOWING MILLIGRAMS OF NON-PROTEIN NITROGEN IN 10 CC. OF MILK DURING DIGESTION BY TRYPSIN AT pH 8.1.

Several experiments were run with trypsin, the results of a typical experiment being given in Fig. 2. The results are averages of duplicate determination.

The results of the work with trypsin are very similar to that with pepsin. The irradiated sample digests more rapidly during the early stages of digestion but finally after 4 hours the non-irradiated sample surpasses it. Here again it should be said that with normal irradiation by the method describes the differences would undoubtedly be very slight if detectable at all.

In explaining the more rapid digestion of the proteins of irradiated milk than of non-irradiated milk by pepsin and trypsin during the early stages of digestion, use may be made of the work of Wallen-Lawrence and Koch[2] who have demonstrated that boiled milk digests more rapidly than unboiled milk due to the destruction of anti-enzymes by boiling. It is possible that irradiation destroys or partially destroys the anti-enzymes in milk thus making irradiated milk more easily digested than milk not so treated.

SUMMARY AND CONCLUSION

A biochemical study has been made of milk and butter from milk treated with a heavy dose of ultraviolet light. Controls of non-irradiated milk and butter were also run. There is very little change in the composition of milk due to irradiation. Butter from irradiated milk has a shorter induction period for oxidation than that from non-irradiated milk. The variation in fat constants is slight. Digestion studies in vitro with pepsin and trypsin indicate a slight speeding up of digestion during the early stages of the process. This may be due to a destruction of anti-enzymes by ultraviolet irradiation. Normal irradiation by the method described would likely produce no detectable change in the composition or digestibility of milk.

ACKNOWLEDGMENTS

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VITAMIN A CONTENT OF PASTURE PLANTS

I. WHITE CLOVER (*Trifolium repens*) AND KENTUCKY BLUE GRASS (*Poa pratensis*) UNDER PASTURAGE CONDITIONS AND FED GREEN

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Vitamin A content of pasture plants deserves consideration, first because so large a proportion of the total feed supply of dairy cows is derived from pastures, and second because the vitamin A content of milk and butter may be influenced by the ration of the cow.

Sherman and Smith (11) suggest that the cow is able to store a surplus of vitamin A in the body during the summer months when fed green plants. This store may then be drawn upon during the winter months when the cow is fed dry feeds.

Under uniform stall feeding conditions McLeod, Brodie, and Macloon (7) found that milk varied little in vitamin A content from season to season. Drummond, Coward, and Watson (2) in 1921 pointed out that the vitamin A content of milk at different seasons is dependent upon the diet. Kennedy and Dutcher (5) found that the amount of vitamins A and B in milk depends upon the abundance of the supply in the feed of the cow.

That the vitamin A content of milk may be raised by the feeding of cod-liver oil, kale, or fresh pasture has been shown by Drummond, Coward, and Watson (2), Luce (6), Chick and Roscoe (1), and Golding, Soames, and Zilva (4). Golding (3) reported butter made from milk produced by cows fed green plants to be ten times as potent in vitamin A as butter made from milk produced by cows fed dry winter feeds.

The University of Idaho has 14 plots of grasses and legumes which are being studied in an effort to formulate desirable pasture mixtures. Data are being taken on growth habits, yield, palatability, and chemical composition. The influence of the cow's food on the vitamin A content of her milk suggested that the different grasses and legumes might vary sufficiently in vitamin A content to be an important consideration in formulating pasture mixtures.

This paper is a preliminary report on the biological analysis of the vitamin A content of white clover (*Trifolium repens*) and Kentucky blue grass (*Poa pratensis*), two of the most common plants used in pasture mixtures.

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EXPERIMENTAL PROCEDURE

The method used for determining the vitamin A content of these plants was based on the technique of Sherman and Munsell (10). Young rats, 28 to 29 days of age, were taken from mothers which had received Sherman's diet No. 13 (⅔ ground whole wheat and ⅓ whole milk powder, plus 2 per cent of the weight of wheat in salt). These rats were placed on a basal vitamin A free diet. This basal diet consisted of 67 per cent corn starch, 18 per cent alcohol-extracted casein, 4 per cent salt mixture (Osborne and Mendel (9)), 10 per cent dried yeast, and 1 per cent salt. Fresh water and the basal vitamin A-free diet were always available.

Vitamins B and G were supplied by feeding dried yeast. One-pound cakes of a commercial yeast were placed in thin layers and allowed to dry at room temperature. When dry the yeast was ground and incorporated into the diet at a 10 per cent level. Vitamin D was furnished by 15 minutes' irradiation of the rats with a sun lamp at 30 inches.

Fresh, green samples of white clover and Kentucky blue grass were received once a week from the University of Idaho experimental plots. The plants were cut into fine bits by scissors. A sufficient number of doses for the week were weighed into one-fourth-ounce ointment jars with screw caps and placed in an electric refrigerator.

Each plant was fed at three levels, 60, 180, and 600 milligrams per week.

Three animals were kept in each cage for the first three weeks of the depletion period. At the end of the third week they were placed in individual cages made of one-fourth inch wire cloth. The bottom of each cage was raised to prevent access to feces.

At the close of the depletion period, determined by the cessation of growth and the appearance of ophthalmia, the rats were placed on experiment and continued for eight weeks.

Each animal was weighed once a week, and the food consumption recorded. Weighed doses of the plants were fed three times a week. Daily observations were made and notes taken on symptoms indicating a lack of vitamin A in the diet and a post-mortem examination was made of each animal.

RESULTS

Animals receiving 60 milligrams of clover per week (figure 1) made an average total gain in body weight of 48 grams during the experimental period of eight weeks. By the beginning of the third week the eyes of the five animals on this level of clover were again normal, and they remained so throughout the experiment. Post-mortem examinations showed that one of the animals had a small amount of pus in the nasal sinuses.

The five animals receiving 60 milligrams of blue grass made an average total gain in body weight of 25.8 grams in eight weeks, which is approxi-

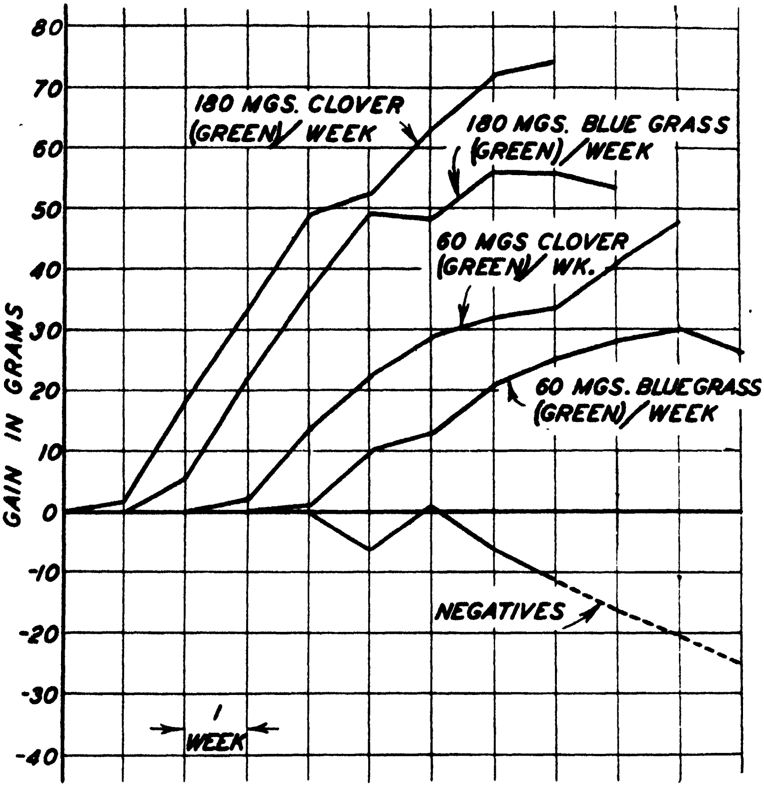

FIG. 1. AVERAGE WEEKLY GAIN OF RATS ON DIFFERENT FEEDING LEVELS OF WHITE CLOVER AND BLUE GRASS.

mately one unit gain.[1] None of the rats in this group recovered from ophthalmia during the experimental period. Post-mortem examinations showed that one of the animals had pus in the nasal sinuses, in the inner ear, and at the base of the tongue. A second animal had pus in the nasal sinuses and at the base of the tongue, while a third had pus in the nasal sinuses only. The remaining two were free from signs of infection.

At the level of 180 milligrams of clover, the rats made an average total gain of 74.6 grams. All signs of ophthalmia disappeared within a period of two weeks. While this gain is below normal, the animals were sleek and thrifty and approximately normal in appearance. Upon post-mortem examination one rat was found to have a small pus sac at the base of the tongue.

Animals receiving 180 milligrams of blue grass made an average total gain of 53.6 grams. While this group of animals lacked the sleek appearance of the animals on the same level of clover, their eyes were cured in the same length of time, and remained so, with the exception of one rat whose eyes showed a distinct narrowing during the eighth week. This animal was found to have pus in the nasal sinuses and at the base of the tongue, and a second rat had pus in the nasal sinuses only.

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[1] The amount of a given food necessary to induce a gain of 3 grams per week for 8 weeks is considered to supply one rat unit of vitamin A.

The two rats receiving 600 milligrams of clover per week made gains of 61 and 63 grams, respectively, while the two receiving the same level of blue grass made gains of 44 and 52 grams, respectively.

The five rats used as negative controls lost an average of 22.6 grams in body weight and survived an average period of 53.6 days. Post-mortem examination showed that four of the five rats had pus in the nasal sinuses and at the base of the tongue.

Computed on a rat unit basis these results indicate that under summer pasturage conditions blue grass contains about 100 units of vitamin A per gram and white clover contains 200.

DISCUSSION

Sherman and Smith (12) say that escarole is the richest source of vitamin A so far reported. They also report one sample of dried alfalfa as containing 100 units per gram. McLaughlin (8) studied the relation of vitamin A content to size of leaves, using New Zealand spinach. She reported a growth of 16 grams in 8 weeks on 70 milligrams per week of the very small leaves. On 90 milligrams of the same sample a growth of 41 grams in 8 weeks was obtained. In terms of vitamin A units these small leaves would contain from 90 to 150 units per gram.

Compared with results reported on other plant tissues, white clover (200 units) would be classed as one of the richest sources of vitamin A, while blue grass (100 units) would appear to be in about the same class as the young New Zealand spinach and the one sample of dried alfalfa.

Experiments are now in progress to determine the loss of vitamin A caused by drying these pasture plants, and it is also planned to continue the study of other pasture plants, both green and dried.

CONCLUSIONS

1. Results of a preliminary study of the vitamin A content of white clover (*Trifolium repens*) and Kentucky blue grass (*Poa pratensis*) have been presented.

2. The results of these experiments indicate that white clover contains at least 200 units of vitamin A per gram and blue grass is about one-half as rich.

3. Comparing these results with those of experiments reported on other plant tissues, white clover would be classed as one of the best sources of

vitamin A, while blue grass would be classed with the best sample of dried alfalfa.

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THE EFFECT OF PROLONGED HOLDING AT PASTEURIZATION TEMPERATURE ON THE PROPERTIES OF AN ICE CREAM MIX*

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INTRODUCTION AND REVIEW OF LITERATURE

This investigation was started as a result of a question raised by the Research Committee of the International Association of Ice Cream Manufacturers, as to what effect prolonged holding of an ice cream mix at pasteurization temperature would have on the properties of the mix and quality of the ice cream.

It is common practice in ice cream plants to pasteurize a batch of mix in a vat, and at the end of the usual holding period of 30 minutes, the mix is homogenized. The homogenization process may require thirty minutes to one hour or longer depending on the capacity of the machine. This means that some portions of the mix will be held for a longer period than other portions. It is also possible to encounter other delays, due to burned out fuses, cutting off of the power or a broken belt, in which case the mix might be held for abnormally long periods. Very little information is available on the effects of prolonged holding of the mix at pasteurization temperature. One phase of this work was studied by Martin, Swope and Knapp (1) who found that holding periods up to two hours, and pasteurization temperatures of 145, 155 and 165° F. had but little effect on the rate of overrun incorporation. The effect of various temperatures and holding periods on the bacteria content of ice cream has been studied by Fay and Olsen (2) and Fabian and Cromley (3) who demonstrated that 150° F. for thirty minutes was sufficient to destroy from 94.5 to 99.9 per cent. of the bacteria present in the mix. Although considerable work has been done on thermophilic bacteria in milk, conditions in an ice cream mix are somewhat different due to the presence of fat and sugar in a greater concentration. Yale (4) found that prolonged holding at pasteurization temperature in the case of milk did favor the development of thermophiles, but faulty plant operation proved to be more important in the development of these organisms than the type of pasteurizing equipment used.

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* The author wishes to acknowledge the valuable assistance given by Glen Aikin, Department of Bacteriology of the Kansas State College, in conducting the bacteriological phases of this problem.

METHODS

A standard ice cream mix, made from unpasteurized sweet cream and skimmilk, sweetened condensed skimmilk, sugar and gelatine, calculated to contain 12, 10, 15 and 0.4 per cent fat, serum solids, sugar and gelatine, respectively, was used in this experiment. The ingredients were mixed together in a glass lined vat and heated to 150° F. and maintained at that temperature. A ten gallon portion of the mix was passed through a homogenizer at 2,500 pounds pressure at the end of 30 minutes, 1.5, 2.5 and 3.5 hours of holding. The first two gallons of mix over the cooler in each lot were discarded to make sure that the lot collected did not contain any mix from the previous batch which might have remained in the homogenizer and pipe lines, and also to make sure that the homogenizer was properly adjusted. The mixes were aged in ten gallon cans for 24 and 48 hours. Samples of the mix were taken in sterile bottles from the vat for bacteria counts, and other samples were taken from each lot of mix for the various determinations. Unpasteurized milk products were used in order to eliminate in so far as possible the effects of any previous heat upon the ingredients, the sweetened condensed skimmilk being the only previously heated milk product used.

Five trials were made, three of which were frozen in a 40 quart brine freezer, and two were made in a 40 quart direct expansion machine. Exactly 40 pounds of mix were used in each run. The brine temperature or suction pressure was kept constant through each trial so as to insure uniform freezing conditions. A preliminary batch was always frozen in order that the freezer would be thoroughly chilled before the experimental batches were frozen. Overrun determinations were made at minute intervals after the first two minutes and at half minute intervals near the end of the freezing process with a Mojonnier overrun tester. The temperature change throughout the freezing process was observed on a Centigrade thermometer graduated in one-tenth degrees. Samples of the ice cream were taken in one-half gallon tin cans, and hardened for quality determinations.

Five samples for bacteriological study were taken during each trial. The first or control sample was taken at the time the mixed ingredients had reached a temperature of 110° F. The remaining samples were taken at the same time the ten gallon portions were removed. The samples were packed in ice as they were taken from the vat and held until all the samples for a trial had been collected. They were then taken to the laboratory where duplicate dilutions of each sample were made as outlined in "Standard Methods of Milk Analysis." One set of the plates was poured with plain agar and incubated for 48 hours at 37° C. The other set was poured with dextrose agar and incubated at 62.5° C. to determine if the prolonged holding at pasteurization temperature might result in the growth of thermophiles. Three trials failed to show any indication of growth of these organisms on the plates, so in the last trials the raw mix was inoculated with a culture of thermophiles to make sure of their presence in the mix.

Samples of the mixes taken for other laboratory studies were tested immediately after collection for viscosity, acidity, and protein stability; these same tests were also made on other samples which were held for 24 and 48 hours. The acid content of the mixes was determined by titrating a 9-gram sample with tenth normal sodium hydroxide, using phenolphthalein for an indicator. Viscosity tests were made with a MacMichael viscosimeter at 25° C., using a No. 27 wire on 3 cm. bob.

The protein stability was measured by a method devised for this purpose. An attempt was made to use the method described by Dahle (5) but it was not entirely satisfactory owing to the difficulty in observing the point of coagulation. Ten cc. of rennet extract and 240 cc. of distilled water were mixed together and 2 cc. of this solution added to 10 cc. of ice cream mix which had been diluted with 10 cc. of distilled water at 100° F. The time required for first signs of feathering was recorded as the coagulating time. The feathering was observed by frequently tilting the contents of the beaker used in this determination and as the mix flowed down the sides the first sign of feathering could be easily determined. This test gave consistent results and appears to have possibilities as a means of measuring the effect of heat on ice cream mixes.

EFFECT OF PROLONGED HOLDING OF A MIX AT PASTEURIZATION TEMPERATURE ON SOME OF ITS PROPERTIES

The titratable acid content of the mixes made in this experiment was very uniform, varying between 0.17 and 0.20 per cent. For the five trials the average acidity for each of the holding periods was found to range between 0.184 and 0.19 per cent with practically no variation between holding periods or between the fresh and twenty-four hour samples.

Samples of mix taken from the various lots immediately after homogenization and after aging twenty-four hours indicated that the prolonged holding had only slightly affected the viscosity. As the holding period was prolonged the average test of the five trials showed a gradual reduction from 26 to 15.2 centipoises on the fresh samples and 59.2 to 47.2 on the aged samples.

The time required for a weak rennet solution to produce the first signs of feathering of the mix was measured. This test indicated that the prolonged holding was changing the stability of the mix. The time required for feathering increased in direct proportion to the length of the holding period. Mixes held at 150° F. for thirty minutes would feather upon addition of the rennet solution in about two minutes, while mixes which had been subjected to heat for 3.5 hours would not show signs of coagulation until 6 to 8 minutes had elapsed after the rennet was added. An attempt was made to determine the protein stability by means of measuring the number of cc. of 95 per cent alcohol and water required to produce coagulation; however, it was discarded in favor of the rennet test due to the inability of accurately judging just when coagulation took place.

TABLE 1

Effect of prolonged holding of a mix at pasteurization temperature on acidity, viscosity, and protein stability of fresh and 24 hours old mixes

(Average of 5 trials)

AGE OF MIX	HOURS HELD AT PASTEURIZATION TEMPERATURE							
	0.5		1.5		2.5		3.5	
	Fresh	24 hrs.	Fresh	24 hrs.	Fresh	24 hrs.	Fresh	24 hrs.
Acidity[1]	0.186	0.19	0.19	0.19	0.184	0.19	0.19	0.19
Viscosity[2]	26.00	59.2	23.8	68.4	21.8	53.6	15.2	47.2
Protein Stability[3]	2.26	3.0	3.12	4.0	4.15	4.8	7.0	6.6

[1] Titrable acidity.
[2] Viscosity in centipoises.
[3] Time in minutes required for first signs of coagulation.

EFFECT OF PROLONGED HOLDING OF A MIX AT PASTEURIZATION TEMPERATURE ON BACTERIA DESTRUCTION

The mixes used in this study were pasteurized in a glass enamel lined jacketed vat, using hot water for the heating medium. The temperature of 150° F. was selected, since it is quite generally used by ice cream plants and is regarded as satisfactory for bacterial destruction. Since this experiment involved the subjecting of the mix to pasteurization temperature for a rather extended period of time, it seemed that there might be a possibility

TABLE 2

Effect of prolonged holding a mix at 150° F. on the standard plate counts of five samples

TRIAL	CONTROL	HELD 30 MIN.	HELD 90 MIN.	HELD 150 MIN.	HELD 210 MIN.
1	9,400,000	4,800	1,900	1,800	2,000
2	8,200,000	6,400	3,500	2,600	3,100
3	5,200,000	3,300	3,200	4,200	3,800
4	6,900,000	10,000	10,000	11,000	9,900
5	22,000,000	30,000	41,000	19,000	3,800
Average	11,000,000	11,000	12,000	8,000	4,500

for the growth of thermophiles during the holding period. No thermophiles were present in the plates made from any of the mixes in the first three trials. In the last two trials the raw mix was inoculated with organisms which had been growing in milk at 150° F., but these organisms also failed to grow on the plates under the specified conditions.

The counts recorded in table 2 indicate that the greatest portion of bacterial destruction due to heating occurred during the first thirty minutes of holding. Extended holding at 150° F. over thirty minutes did not materially lower the standard plate count in any of the five trials.

EFFECT OF PROLONGED HOLDING OF A MIX AT PASTEURIZATION TEMPERATURE ON RATE OF OVERRUN INCORPORATION

Prolonged holding of the mixes at 150° F. apparently had no effect on the rate of overrun incorporation. Results obtained on twenty-one mixes held 24 and 48 hours, respectively, before freezing, showed that mixes held in the pasteurizer for 3.5 hours froze as rapidly as those held for shorter periods. In table 3 the time required to reach 100 per cent swell is recorded. This method of recording overrun might be subject to some criticism since it is difficult to determine at what interval the 100 per cent swell is obtained, as it is impossible to take samples less frequently than one every thirty seconds.

TABLE 3

Minutes required to obtain 100 per cent swell in mixes held for a prolonged period at pasteurization temperature

TRIAL NO.	HOURS HELD AT PASTEURIZATION TEMPERATURE			
	0.5	1.5	2.5	3.5
	Mixes aged 24 hours			
1	11.0	10.0	9.5	9.5
2	13.5	13.5	13.0	13.5
3	9.0	9.5	9.5	9.0
4	12.0	11.0	11.0	11.0
5	10.0	10.5	12.0	11.0
Average	11.1	10.9	11.0	10.8
	Mixes aged 48 hours			
1	9.0	8.0	10.0	10.0
2	11.5	12.0	12.0	11.5
3	8.5	8.0	8.5	8.5
4	10.5	9.5	11.0	12.0
5	11.0	10.5	10.0	10.0
Average	10.1	9.6	10.3	10.2
Average of 24 & 48 hours	10.6	10.25	10.65	10.50

Another way of expressing overrun is to record the amount obtained at the end of a certain period. In table 4 are recorded the overruns obtained at minute intervals after the first minute. It shows the rate of overrun incorporation as well as the amount obtained at the end of a 10 and 9 minute interval, respectively, for mixes aged 24 and 48 hours. When this method of expressing overrun was used, the results are slightly different from those expressed in table 3. The 48 hour mixes froze somewhat faster than the 24 hour mixes, however the prolonged heating did not seem to impair the rate of overrun except possibly to a slight extent in the 48 hour aged mixes, which on the average did not whip as fast in case of the 3.5 hours of holding when compared with 0.5 or 1.5 hours of holding.

TABLE 4

Effect of prolonged holding of mixes at pasteurization temperature on rate of overrun incorporation Data in per cent overrun

MINUTES	Hours held at 150° F.							
	AGED 24 HOURS				AGED 48 HOURS			
	0.5	1.5	2.5	3.5	.5	1.5	2.5	3.5
2	32.5	36.2	37.0	39.0	36.5	40.2	36.8	37.1
3	41.0	43.4	47.0	47.8	45.6	49.4	47.4	48.1
4	50.0	53.0	54.0	56.8	53.2	57.7	58.1	58.7
5	58.0	57.0	63.0	62.2	61.7	65.3	64.9	64.9
6	66.6	67.0	69.0	68.6	68.9	73.3	70.7	71.1
7	73.8	74.8	74.4	74.8	75.7	80.3	76.7	75.3
8	80.2	82.6	80.0	81.6	83.3	89.1	80.8	80.7
9	87.4	89.0	92.2	88.8	95.1	99.5	91.5	91.1
10	96.4	97.0	97.8	97.6				

EFFECT OF PROLONGED HOLDING ON THE QUALITY OF THE ICE CREAM

In only two instances did the judges detect any heated flavor in the finished ice cream, and then it was not serious. All the samples of ice cream were of excellent quality. The body and texture of the ice cream was equally good for the samples regardless of the length of the holding period as shown in table 5.

DISCUSSION AND SUMMARY

Five trials each consisting of four mixes were made in this experiment. Freezing was made of mixes held for 24 and 48 hours making forty individual freezings.

The results of this experiment indicate that ice cream mixes can be heated to 150° F. and held at that temperature for 3.5 hours without impairing the whipping properties or quality of the ice cream. Other

TABLE 5

Effect of prolonged holding at pasteurization temperature on the flavor and body and texture score of the ice cream made from mixes aged 24 hours

TRIAL NO.	HOURS HELD AT PASTEURIZATION TEMPERATURE							
	0.5		1.5		2.5		3.5	
	F	B	F	B	F	B	F	B
1	45.0	23.0	45.0	23.0	45.0	23.0	45	23.0
2	45.0	23.5	45.0	23.5	45.0	23.5	45	24.0
3	47.0	24.5	47.0	24.5	47.0	24.0	45*	24.0
4	45.0	24.0	45.0	24.0	46.0	24.0	45	24.0
5	46.0	24.0	46.0	24.0	46.0	24.0	45*	24.0
Average	45.6	23.8	45.6	23.8	45.6	23.7	45	23.8

F—Flavor score, perfect = 50.
B—Body and Texture score, perfect = 25.
* Slight heated flavor.

properties such as viscosity and protein stability are affected to some extent, the tendency being for a slight decrease in viscosity and an increase in the protein stability as the holding period is prolonged. From a bacteriological standpoint these trials would indicate that no serious trouble would be encountered from heat loving bacteria as determined by the plate count given in standard methods, provided the processing was properly done.

No attempt was made at a direct microscopic bacterial count; the results obtained were by the plate count method. The majority of the bacteria were destroyed at the end of a 30 minute holding period, the reduction being only slight after that time.

REFERENCES

(1) Martin, W. H., Swope, W. D., and Knapp, I. R. Problems in the manufacture of ice cream—Pasteurization temperatures. Penn. Agr. Expt. Animal Report 38, Bul. No. 196, 20–21. 1925.

(2) Fay, A. C., and Olson, N. E. The bacterial content of ice cream. Journal of Dairy Science 7: 330–336. 1924.

(3) Fabian, F. W., and Cromley, R. H. The influence of manufacturing operations on the bacterial count of ice cream. Mich. Agr. Exp. Station Tech. Bul. No. 60. 1923.

(4) Yale, N. W. The control of thermophilic bacteria in pasteurizing plants. Jour. Bact. 19: No. 1, 46–47. 1930.

(5) Dahle, C. D., and Barnhart, G. S. The effect of pasteurizing and homogenizing. Temperature on certain properties of ice cream mixes. Jour. Agr. Res. 42: No. 10, 678. 1931.

PROCEEDINGS OF THE 27TH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The 27th annual meeting of the American Dairy Science Association was held at the College of Agriculture, University of Kentucky, Lexington, on June 28th to 30th. The attendance was particularly pleasing to those in charge of the meeting for the registration showed approximately 200 men and 80 women. It is evident that there is an increased tendency for members to attend the meeting as a part of a vacation trip.

On the afternoon preceding the day of the opening of the meeting a conference on judging dairy products and a conference on judging dairy cattle were held in the dairy building. Special trips were taken by the ladies to see point of historic interest and to visit Berea College. On the second afternoon of the meetings both the men and women enjoyed a tour of the famous horse breeding establishments in the vicinity of Lexington.

For the second consecutive year the program committee prepared abstracts of all papers presented at the meeting which were purchased by attending members for use during the sessions and for later reference. Additional copies of these abstracts are still available and may be secured from Prof. Fordyce Ely, University of Kentucky, Lexington, at the regular price of $1.00 each.

The following program was presented.

GENERAL SESSION

Tuesday Morning, June 28, 1932—Memorial Hall

E. L. ANTHONY, President, American Dairy Science Association, presiding

Registration—Memorial Hall—University of Kentucky Campus.

Call to order, President E. L. Anthony.

Address of Welcome, Dean Thomas P. Cooper, College of Agriculture, University of Kentucky.

President's Address, E. L. Anthony, Michigan State College.

The Journal of Dairy Science, A. C. Dahlberg, Editor, New York Experiment Station, Geneva, New York.

Color of Milk as Affecting its Marketability, H. A. Ruehe and H. K. Wilson, University of Illinois.

Production, Merchandizing and Uses of Supreme Cream, Herman D. Wendt, West Chester, Pennsylvania.

Factors Influencing the Formation of Milk Layer in Bottled Coffee Cream, J. L. Mooney and L. H. Burgwald, Ohio State University.

Possibilities of Employing a More Simplified and Direct Method of Grading Creamery Butter, Roy C. Potts and C. E. Eckles, Bureau of Agricultural Economics, U. S. D. A.

A Method for Studying the Question of Stripping Following Machine Milking, W. E. Peterson and Phillip Swenson, University of Minnesota.

Soft Curd Milk, H. B. Monier and H. H. Sommer, University of Wisconsin.

The Effect of the Cow's Ration on the Vitamin (B and G) Content of Milk, W. E. Krauss, Ohio Agricultural Experiment Station.

The Use of Cleansers on Metal Dairy Equipment, P. S. Lucas, Michigan State College.

Announcement of Committee Appointments.

GENERAL SESSION

Tuesday Afternoon, June 28, 1932—Memorial Hall

A. C. Dahlberg, Journal of Dairy Science, Presiding

The Development of the Mammary Gland.

a. The Growth of the Gland, C. W. Turner, A. H. Frank and A. B. Schultze, University of Missouri.

b. The Initiation of Secretion, C. W. Turner and W. U. Gardner, University of Missouri.

The Manufacture and Use of Low Lactose Skim Milk, Byron H. Webb and O. E. Williams, Bureau of Dairy Industry, U. S. D. A.

The Electrokinetic Potential at the Fat-Milk Serum Interface and its Relation to Creaming Ability, G. C. North and H. H. Sommer, University of Wisconsin.

Some Studies on the Commercial Irradiation of Milk, K. G. Weckel and H. C. Jackson, University of Wisconsin.

The Cultivation of Lactobacillus Acidophilus, Paul W. Allen, University of Tennessee.

Sterilization of Dairy Equipment by Dry Heat, J. C. Marquardt, New York Agricultural Experiment Station.

Reading Babcock Tests, Thos. B. Harrison, University of Tennessee.

The Effect of Viscolization on Some of the Physical Properties of Milk, C. P. Halloran and G. Malcolm Trout, Michigan State College.

Cultured Cream, E. S. Guthrie, Cornell University.

Does the Metal Content of Milk Contribute to the Corrosion of Dairy Equipment? H. A. Trebler, F. L. LaQue and W. A. Wesley, Research Laboratories, National Dairy Products Corporation.

The Effect of Certain Methods of Protein Precipitation upon the Polarimetic Determination of Lactose in Milk, John T. Poffenberger and F. E. Almy, Ohio State University.

EXTENSION PROGRAM

Tuesday Afternoon, June 28, 1932—Room 128 McVey Hall

M. J. Regan, Section Chairman, presiding

Adapting Dairy Extension Methods to Present Conditions, Floyd Johnston, Iowa State College.

Presenting and Using Commodity Outlook Reports, Ivan McKellip, Ohio State University.

The Quality Milk Project, M. L. Flack and P. A. Downs, University of Nebraska.

Methods of Presenting Pasture Management Information, M. J. Regan, University of Missouri.

Report of Committee on Feeding, L. A. Higgins, Mississippi A. and M. College.

Dairy Extension Plans of Work in Marginal Dairy Territory, Ted Besh, University of Kentucky.

Methods of Presenting Better Sires Work—Evaluating and Locating Better Sires, S. J. Brownell, Cornell University.

Report of Better Sires Committee, Jas. W. Linn, Kansas State Agricultural College.

Changing the Method of Summarizing the D. H. I. A. Records from a ''cow year'' to a ''cow unit'' basis, A. C. Baltzer, Michigan State College.

Report of committee on suggested changes in D. H. I. A. Forms, C. R. Gearhart, Pennsylvania State College.

Business Meeting.

COLLEGE INSTRUCTION PROGRAM

Tuesday Afternoon, June 28, 1932—Room 111 McVey Hall

H. P. Davis, University of Nebraska, presiding

The Organization, Content and Presentation of the First or Orientation Course in Dairy Husbandry, L. K. Crowe, University of Nebraska.

The Demonstration vs. the Laboratory Method in Teaching Elementary Courses in Dairying, C. H. Eckles, University of Minnesota.

The Value of Abilities and Understandings in the Dairy Husbandry Course, C. Y. Cannon and L. B. Harmon, Iowa State College.

The Utilization of the College Herd and Farm for Teaching Purposes, Chas. N. Shepardson, Texas A. and M. College.

The Place of the Practical Course in the Dairy Curriculum, Earl Weaver, Oklahoma A. and M. College.

Building a Curriculum in Dairy Technology, R. B. Stoltz, Ohio State University.

A Report on Some Means which the Dairy Industry Department at Iowa State College has Recently Used to Attain Improved Teaching Methods, E. F. Goss, Iowa State College.

The Project Method of Teaching the Market Milk Course, G. Malcolm Trout, Michigan State College.

Should College Dairy Plants Operate Commercially? H. A. Ruehe, University of Illinois.

DAIRY MANUFACTURES PROGRAM

Wednesday Morning, June 29, 1932—Memorial Hall

R. B. Stoltz, Section Chairman, presiding

Microbiology of Butter, E. S. Guthrie, C. N. Stark and Pauline Stark, Cornell University.

The Influence of the Medium on the Yeast and Mold Count of Butter, E. H. Parfitt, Purdue University.

Chemical and Bacteriological Studies of Oklahoma Butter, E. L. Fouts and J. I. Keith, Oklahoma A. and M. College.

Some Studies Made on the Quality and Composition of Butter made from Plastic Cream, J. B. Linneboe, University of Wisconsin.

Influence of Various Factors in the Manufacturing Process on Gumminess and other Body Characteristics of Southern Butter, William White, Bureau of Dairy Industry, U. S. D. A.

Studies on Defects of Unsalted Butter, E. O. Herreid and H. Macy, University of Minnesota.

Phospholipids in Milk. Their Effect upon Fat Analysis of Skim Milk and Buttermilk, P. A. Wright, E. F. Deysher and G. E. Holm, Bureau of Dairy Industry, U. S. D. A.

Studies on the Freezing of Milk and Cream, Bruce Baldwin and W. B. Combs, University of Minnesota.

Factors in the Age Thickening of Sweetened Condensed Milk, V. C. Stebnitz and H. H. Sommer, University of Wisconsin.

The Use of Corn Sugar in the Manufacture of Sweetened Condensed Milk, P. H. Tracy, University of Illinois.

Business Meeting.

DAIRY PRODUCTION PROGRAM

Wednesday Morning, June 29, 1932—Room 111 McVey Hall

G. BOHSTEDT, Section Chairman, presiding

The Vitamin A and D Requirements of Dairy Calves, I. W. Rupel, G. Bohstedt and E. B. Hart, University of Wisconsin.

A Study of the Anti-Rachitic Requirements of Calves,—The Anti-Rachitic Potency of Oat Straw, Dehydrated Hay and Semi-dried Hay, K. G. Landsburg and S. I. Bechdel, Pennsylvania State College.

Seasonal Composition of Pasture Grasses, R. H. Lush, Louisiana State University.

Dietary Acidosis in Dairy Cattle. A study of the results of feeding large amounts of high acid silage, acids, and single feeds and feeds in combination as measured by blood and urine composition, A. E. Perkins and C. F. Monroe, Ohio Agricultural Experiment Station.

Bone Meal and Marble Dust as Mineral Supplements to the Dairy Ration, W. H. Eaton, Alabama Polytechnic Institute.

Physiological Effects of Rations with Low Grade Roughage, E. B. Meigs and H. T. Converse, Bureau of Dairy Industry, U. S. D. A.

The Phosphorus Requirements of Milking Cows when Alfalfa Furnishes the Principle Source of Protein, C. F. Huffman, C. W. Duncan and L. W. Lamb, Michigan State College.

Calcium Deficiency in Growing Cattle, T. W. Gullickson and C. H. Eckles, University of Minnesota.

Comparison of the Blood Sugar Level of the Mammary and Jugular Veins of Dry and Lactating Cows, H. A. Herman and C. W. Turner, University of Missouri.

The Influence of Age of Cows and Age of Bulls on the Number of Services Required for Conception, R. F. Morgan and H. P. Davis, University of Nebraska.

Business Meeting.

WEDNESDAY AFTERNOON, JUNE 29, 1932

1:30 at Memorial Hall

Tour of Blue Grass Horse Farms and Estates.

C. V. Whitney Farm, Green Tree Farm, Elmendorf Farm, Dixiana Farm, Faraway Farm, Walnut Hall Farm, Coldstream Farm, Idle Hour Farm.

6: 30 Convention Banquet.

GENERAL SESSION

Thursday Morning, June 30, 1932—Memorial Hall

E. L. ANTHONY, President, American Dairy Science Association, presiding

The Effect of Leaving Milk in the Udder at Each Milking on Persistency of Yield, T. E. Woodward, Bureau of Dairy Industry, U. S. D. A.

The Digestibility of Fresh Whole Milk, Evaporated Milk and Powdered Whole Milk, D. D. Shaw and W. B. Nevens, University of Illinois.

The Digestibility of Milk *in vivo* as Affected by Certain Physical Treatments, C. Y. Cannon, D. L. Espe and F. N. Mortensen, Iowa State College.

Some Reactions of the Dairy Cow to Changes in Environmental Temperature and Humidity, W. M. Regan and G. A. Richardson, University of California.

Soybeans—Some of their Chemical and Physical Effects on Milk and Butterfat, J. H. Hilton, Purdue University.

Normal Growth of Dairy Cattle, J. B. Fitch, H. W. Cave and W. H. Riddell, Kansas State Agricultural College.

Laboratory Studies on the Chemistry of Soft Curd Milk, Samuel M. Weisberg, E. V. McCollum and Arnold H. Johnson, Research Laboratories, National Dairy Products Corporation.

A Comparison of the Milk Fat Values of Butterfat from Four Different Breeds of Dairy Cattle on Similar Feed, T. S. Sutton, Ohio State University.

Business Meeting.

DAIRY MANUFACTURES PROGRAM

Thursday Afternoon, June 30, 1932—Memorial Hall

R. B. STOLTZ, Section Chairman, presiding

Some Factors Influencing the Crystallization of Lactose in Ice Cream, Randall Whitaker, Research Laboratories, National Dairy Products Corporation.

The Use of Vegetable Stabilizers in Ice Cream, W. J. Caulfield and W. H. Martin, Kansas State Agricultural College.

New Developments as Regards the Behavior of Gelatin in Ice Cream, W. S. Mueller, Massachusetts State College.

Ash Content of Cocoa and its Influence on the Whipping Ability of Chocolate Ice Cream, Vincent Reeves and J. H. Erb, Ohio State University.

Prolonged Holding of an Ice Cream Mix at Pasteurization Temperature, W. H. Martin, Kansas State Agricultural College.

Limitations of the Dairy Science Score Card for Ice Cream, A. D. Burke, Alabama Polytechnic Institute.

Studies on Whipping Cream, Hugh L. Templeton, University of Wisconsin.

Factors Governing the Manufacture of Sweet Curd Cottage Cheese, L. M. Thurston, West Virginia University.

A Study of Cottage Cheese Made with Rennet, Martin F. Kelly and Walter V. Price, University of Wisconsin.

Some Developments in the Commercial Manufacture of (Philadelphia) Cream Cheese, E. L. Reichart, University of Nebraska.

Relation between Titrable Acidity, Hydrogen Ion Concentration and Quality of Cheddar Cheese during the Making and Ripening Process, L. W. Brown and Walter V. Price, University of Wisconsin.

DAIRY PRODUCTION PROGRAM

Thursday Afternoon, June 30, 1932—Room 111 McVey Hall

G. BOHSTEDT, Section Chairman, presiding

Cottonseed Meal in the Ration of Calves Under Six Months of Age, J. P. LaMaster, Clemson Agricultural College.

The Nutritional Value of Cottonseed Meal as Affected by the Absence of Various Dietary Factors, S. I. Bechdel and R. S. Williams, Pennsylvania State College.

Some Physiological Effects of Gossypol, L. A. Moore, Michigan State College.

Wheat in the Dairy Ration. Its Effect on the Cow, Production and Product. Two Experiments, C. F. Monroe and C. C. Hayden, Ohio Agricultural Experiment Station.

The Value of Oat Feed when Fed in Grain Mixtures and as a Hay Substitute for Dairy Cattle, G. Bohstedt, A. W. Lathrop and A. H. Walker, University of Wisconsin.

Types and Varieties of Corn for Silage in the Corn Belt. Preventing Surface Losses in Silo, W. B. Nevens, University of Illinois.

Soybeans for Silage, R. B. Becker and W. M. Neal, Florida Agricultural Experiment Station.

Methods for Testing Palatability of Roughages and some Preliminary Results, C. H. Eckles, University of Minnesota.

The Feeding Value and Effect of Mint Hays, George E. Taylor, Michigan State College.

A Score Card for Production Records of Dairy Cows, C. E. Wylie, University of Tennessee.

Lactation Revords vs. 12 Months Records for Proving Dairy Bulls, J. C. McDowell, Bureau of Dairy Industry, U. S. D. A.

More Evidence on Proved Sires, R. R. Graves, Bureau of Dairy Industry, U. S. D. A.

MINUTES OF THE EXECUTIVE COMMITTEE MEETING ON THE EVENING OF JUNE 27, 1932, LEXINGTON, KY.

E. L. ANTHONY, *President, presiding*

Those in attendance at meeting:—

R. B. STOLTZ, Vice President.
A. C. DAHLBERG, Editor.
G. BOHSTEDT, Chairman of Production Section.
T. W. GULLICKSON, Sec. of Production Section.
M. J. REGAN, Chairman of Extension Section.
ROY C. POTTS, Secretary of Dairy Economics Section.
R. B. BECKER, Chairman of Southern Section.
E. S. GUTHRIE, Chairman of Eastern Section.
R. R. GRAVES, Sec.-Treasurer.

Becker, of Florida, presented the following resolution to the Executive Committee:

"I move that the author, or one of the authors, of each paper presented on the program of the American Dairy Science Association meeting, with the exception of invited speakers, be a member of the association in good standing."

This motion was adopted and it was the consensus of the Executive Committee that this also apply to the meetings of the Divisions of the American Dairy Science Association.

A motion was also adopted to the effect that the Committee on Standardization be permitted to utilize the funds accrued from the sale of separates to publish new standards.

The following committee to work with the Bureau of Standards on the standardization of dairy containers was approved:

PROF. M. MORTENSEN, of Iowa.
PROF. R. B. STOLTZ, of Ohio.
WM. WHITE, Bureau of Dairy Industry.

Professor Stoltz moved that the association invest its present savings account and any additional funds, that in the judgment of the Secretary-Treasurer are not needed for current expenses, in government securities.—Adopted.

The appointment of an Auditing Committee, consisting of J. C. McDowell and R. W. Bell, of the Bureau of Dairy Industry, was approved.

Regan, of Missouri, made a motion that a financial statement be sent each member of the Executive Committee, when the report is audited at the end of the year. Motion carried.

A Nominating Committee, consisting of C. H. Eckles, Chairman, H. O. Henderson, O. E. Reed, Fordyce Ely and Roy Harris, was approved.

A Resolution Committee, consisting of H. B. Davis, Chairman, Earl Weaver, C. E. Wylie, H. W. Gregory, and Ted Besh, was approved.

The report of the Editor was approved and the report of the Secretary-Treasurer was also approved.

The reappointment of Dr. Otto Hunziker to the Journal Management Committee for a period of three years was approved.

C. H. Eckles, Chairman of the Committee on Constitution, discussed with the Executive Committee, the changes in constitution that will be required to meet the requirements of the laws of the District of Columbia under which the association is incorporated.

Professor Guthrie moved that the Committee on Constitution consider a method for the nomination of officers in such manner that the Vice President automatically becomes the President of the association. This motion was carried.

Invitations were received for the 1933 meeting from the following:

University of Minnesota.
Cornell University.
Pennsylvania State College.
Purdue University.
University of Illinois.
Ohio University.

There was some sentiment in favor of the 1933 meeting being held as close to Chicago as possible in order that the members might attend the Century of Progress Exposition, either before or after the American Dairy Science Association meeting.

The choice of the Executive Committee in regard to the place of the 1933 meeting will be announced in the near future.

There was some informal discussion relative to memorials in the JOURNAL for deceased members of the association.

A. C. Dahlberg was appointed Editor and R. R. Graves was appointed Secretary-Treasurer.

MINUTES OF THE BUSINESS MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION, AT LEXINGTON, KENTUCKY, JUNE 30, 1932

Report of Production Section approved.

Report of Manufacturing Section approved.

Report of Extension Section approved.

Executive Committee Report approved.

A motion had been adopted in the Manufacturing Section to the effect that a committee be appointed by the Executive Committee to consider publication of abstracts in the JOURNAL. This motion was defeated by the

Dairy Science Association meeting as a whole. It was then moved by Stoltz, of Ohio, that the above recommendation of the Manufacturing Section be referred to the Committee on Journal Management. This motion was carried.

Professor A. A. Borland moved a vote of thanks to Professor Ely and his co-workers for the preparation of the abstracts of papers of the 1932 meeting. Carried.

Professor Ragsdale, of Missouri, reported on changes in plan for dairy improvement contests. The Executive Committee had previously decided that this committee on Dairy Improvement Contests would be carried forward but that its future would depend upon the renewal of the National Dairy Show.

C. H. Eckles, Chairman of the Committee on Elections, submitted the following nominations:

For President and member of Board of Directors for one year:
  E. L. ANTHONY, Michigan.
  H. C. JACKSON, Wisconsin.
For Vice-President and member of Board of Directors for one year:
  R. B. STOLTZ, Ohio.
  EARL WEAVER, Oklahoma.
For members of Board of Directors for one year term:
  C. L. ROADHOUSE, California.
  C. R. GEARHART, Pennsylvania.
For two year term:
  H. B. ELLENBERGER, Vermont.
  O. HUNZIKER, Illinois.
For three year term:
  L. A. ROGERS, District of Columbia.
  H. A. RUEHE, Illinois.

Professor C. E. Wylie, in the absence of Professor Davis, presented the report of the Resolutions Committee, which follows:

1. Resolved that the American Dairy Science Association express their appreciation to Professor Fordyce Ely, members of his staff, the officers of the University of Kentucky, Lexington Board of Commerce, the Kentucky Dairy Products Association, as well as other individuals and concerns who have cooperated in furnishing the splendid provisions for the comfort and entertainment of the twenty-seventh annual meeting of the American Dairy Science Association.

2. Resolved that the Association express to the Program Committee consisting of Fordyce Ely, C. L. Roadhouse and Martin Mortensen, their appreciation of the preparation of the program and the printed abstracts which have been so helpful to their members, also to H. B. Morrison and Miss Nellie Lawrence who edited the abstracts.

3. Resolved that the Association commend A. C. Dahlberg, editor, and the Journal Management Committee of the JOURNAL OF DAIRY SCIENCE, for the splendid work in improving and enlarging the activities of the JOURNAL OF DAIRY SCIENCE.

4. Resolved that the Association express to President and Mrs. McVey their gratitude for the delightful garden party given for the members and their wives.

5. Resolved that the Association express to Mrs. Ely and her co-workers its appreciation for the numerous and delightful entertainments provided for the ladies.

6. Whereas, the Dairy, Ice Cream and Machinery Supply Association have shown a real interest in our dairy products students' judging contest held annually at the National Dairy Industry Exposition and have furnished financial aid for conducting the contest and funds for fellowship for winners in the contest, be it resolved that the Association express their appreciation for this splendid interest and support.

O. E. Reed was reappointed as representative of the American Dairy Science Association to the National Research Council.

Roy C. Potts, of the Bureau of Agricultural Economics, U. S. D. A., spoke in favor of development of an economics program as a part of the annual program.

Professor Ruche, of Illinois, spoke in favor of the developments of the college teaching section as a part of the program for the National Meeting.

O. E. Reed, of the Bureau of Dairy Industry, U. S. D. A., reported on activities as representative of the association to the National Research Council meetings.

Professor Fitch, of Kansas, and Professor Wylie, of Tennessee, spoke in favor of honoring members at the National meeting, who have rendered distinctive service to the dairy industry.

Professor Mortensen, of Iowa, moved that a committee be appointed to investigate the basis on which certificates should be granted by colleges for non-collegiate work in dairying. This motion was carried.

ACTIVE MEMBERSHIP OF AMERICAN DAIRY SCIENCE ASSOCIATION

September 1, 1932

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